Glyceryl Trinitrate and Toe–Brachial Indexes in Pedal Ischaemia

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Authorship and Access

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person or material that to a substantial extent has been accepted for the award of any other degree or diploma at Charles Sturt University or any other educational institution, except where due acknowledgement is made in the thesis. Any contribution made to the research by colleagues with whom I have worked at Charles Sturt University or elsewhere during my candidature is fully acknowledged.

I agree that this thesis be accessible for the purpose of study and research in accordance with the normal conditions established by the Executive Director, Library Services, Charles Sturt University or nominee, for the care, loan, and reproduction of theses subject to confidentiality provisions as approved by the University.

______________________________

Sylvia McAra

Date: __________________________
I would like to thank the many people involved in the multiple facets of this work. There has been a general spirit of profound generosity, contribution, and hope brought to the project. With the shared aim of making a difference in the lives of people limited by treatable and preventable foot pathology, a common vision prevailed to promote the quality and duration of active, independent, and healthy living. “If not for myself, then for others unknown” was a sentiment common among the participants and contributors.

My role has been as a conduit to imagine this project, engage this communal spirit, enrich it with the science that has been provided by previous scholarship, and extend the boundaries of knowledge with responsible enquiry. This thesis is the culmination of that undertaking. I was highly privileged to have this opportunity, and this assistance, from so many people. The provision of the Compacts federally funded scholarship allowed for the focus and dedication of time over 3 and a half years that enabled the successful completion of this project, which would not otherwise have been possible.

Contributors are too many to mention individually but they include the 100+ study participants; Charles Sturt University media, particularly Wes Ward; the Albury Wodonga Diabetes Support Group for their help with recruitment for the study; the local general medical practitioners for their support and cooperation; Dorevitch Pathology services manager Zennon McCarty; the senior podiatry students of 2011 and 2012 who assisted in data collection for the pilot study; the staff of the podiatry clinics of CSU, most notably Lyn Ewart and Darren Pickersgill; Wendy Rose Davidson and David Britt for data entry; podiatry colleagues Karen Eddy, Kristy Robson, Michelle Hey, Amy Klein, Dr Caroline Robinson, and Dr Karl Landorf; supporters in academic writing, Cassily Charles, Beverly McVilly, and Judy Redmond; for help with statistics, Dr Melissa Nott, Colin Glanville, Prajwal Gyawali; for emotional and practical friendship and support, Geoff Simmons, Therese Schmid, and my family, the nearest and dearest ones, my children, Daniel and Samantha, my parents, Gloria and Peter McAra, and my personal “bon vivant”, Richard Kelly.

Specific guidance was provided by Dr Gayle Smythe, Associate Dean of Research CSU, Dr Ross Richards, and Dr Herbert Jelinek. Specialist assistance was provided by
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Statistical Assistance

Statistics is a subject in which I am not adept, and although this project has widened my horizons and markedly increased my understanding of statistics, I acknowledge I was heavily reliant on the knowledge and expertise of specialists in this area.

Dr Ken Russell provided preliminary advice and assistance with study set-up, and analysis of the pilot study data.

Sharon Neilson reconfigured the original Excel data file for the main study so that the data would be more amenable to analysis for most of the research questions. Sharon was paid for this assistance through her role at the CSU Quantitative Consulting Unit.

Dr Robert Trevethan provided the expertise to select, perform, and report the statistical analyses in Chapter 5. He very kindly pursued these valuable processes, providing his services pertaining specifically to the results section of Chapter 5, at no charge, for many reasons including his deeply altruistic values involving his interest in and understanding of the worth of the project.
Professional Editorial Assistance

Paid editorial assistance was obtained from Dr Robert Trevethan. It was under the recommendation and suggestion of colleague and CSU academic administrator, Dr Julia Coyle, and with the approval of my supervisors that I chose the editor. The editorial service was funded by the operating funds in my Complots scholarship.

Neither the editor’s current nor his former area of academic specialisation is similar to that of mine. There were innumerable exchanges and discussions—sometimes vigorous—about the merits of various and alternative wordings. The nature of the editorial assistance was rich with exemplars, which educated me to immeasurably improve my academic skills, enhance my prowess in analytical thinking, and upgrade my writing in its structure, style, and substance. My greatest hope and satisfaction is that my skills can now serve in dissemination of this work.
Ethics Approval

Permission to conduct this research was sought from, and granted by, Charles Sturt University’s Human Research Ethics Committee, approval number 2011/146. See Appendix A.
Publications

The items listed below are abstracts of presentations at the Australasian Podiatry Conference, Melbourne, in April 2011. These abstracts were published in the *Journal of Foot and Ankle Research* later that year. The first and third items were poster presentations, and the second item was a paper presentation.


This presentation was awarded the prize for the best nonresearch paper at the conference.

Abstract

Glyceryl trinitrate (GTN) has a long history in medicine as a safe and reliable vasodilator. However, it has not yet been established for use in peripheral arterial disease (PAD) despite low pedal blood pressures being associated with foot ulceration and amputation.

There were two primary foci of this research. The first was to determine whether GTN could ameliorate subnormal pedal blood pressure. The second was to determine whether pedal blood pressure could be measured reliably and validly via the toe brachial index (TBI) and to identify some of the characteristics of that index given that it might not only provide an accurate indication of distal pedal blood pressure but also appears to be capable of providing an early warning sign of cardiovascular disease.

The potential of GTN to redress pedal ischemia emerged after rapid healing was observed in four cases of chronic foot ulcer using GTN patches. A review of the literature indicated that GTN was associated with improvements in vascular and neurological function, particularly in warmer temperatures, so a pilot study was conducted to assess the most effective ways of measuring the relevant variables.

Subsequently, 100 people with subnormal TBIs (58 men, 42 women; mean age 69.8 years, SD = 9.5), 58% of whom were people with diabetes, completed a 6-month trial, comprising two intervention groups using 1.25 mg and 2.5 mg of GTN as *Nitro-Dur* (n = 33 and 24 respectively), a placebo group (n = 22), and a control group (n = 21).

Detailed analysis of TBIs was necessary as a precursor to the main analyses. This included identification of the readings that would produce the most valid single TBI, the extent to which TBIs differed between the two feet and were stable over time, and, the relationships of TBIs with demographic and health variables. The effect of GTN on TBIs was analysed per protocol using
ANCOVAs. At 1 month post intervention, the high GTN dose group had significantly higher TBIs compared with both the low GTN dose group and the control group, \( p = .046 \) and \( .020 \); effect sizes of .676 and .822 respectively. After 5 months, both GTN dose groups had significantly higher TBIs compared with the control group \( p = .048 \) and \( .044 \); effect sizes of .531 and .662 respectively. These findings indicate that transdermal GTN at these low doses could be valuable in ameliorating pedal ischemia.

Results from the placebo group complicate these otherwise positive outcomes. Placebo group’s TBIs were between the TBIs of the other three groups at 1 month, and at that time were not significantly different from any of those three groups. However, at 5 months the placebo group was similar to both intervention groups in having higher TBIs relative to the control group \( p = .006 \), effect size = .932).

As additional outcomes of this research, guidelines were produced for determining individual doses of GTN, and an evidence-based vascular assessment flowchart was created to enable appropriate diagnosis, referral, and stratification of risk status for PAD and cardiovascular disease.
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<td>ABI</td>
<td>Ankle brachial index - synonymous with ABPI</td>
</tr>
<tr>
<td>ABPI</td>
<td>Ankle brachial pressure index – synonymous with ABI</td>
</tr>
<tr>
<td>ADL</td>
<td>Activities of daily living</td>
</tr>
<tr>
<td>AF</td>
<td>Atrial fibrillation</td>
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<tr>
<td>BGL</td>
<td>Blood glucose level</td>
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<tr>
<td>BP</td>
<td>Blood pressure</td>
</tr>
<tr>
<td>BK</td>
<td>Below knee</td>
</tr>
<tr>
<td>BKA</td>
<td>Below knee amputation</td>
</tr>
<tr>
<td>CAD</td>
<td>Carotid artery disease</td>
</tr>
<tr>
<td>cGMP</td>
<td>Cyclic GMP</td>
</tr>
<tr>
<td>COAD</td>
<td>Chronic obstructive airways disease</td>
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<tr>
<td>CT</td>
<td>Connective tissue disease</td>
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<tr>
<td>CVA</td>
<td>Cerebrovascular accident</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
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<tr>
<td>CVT</td>
<td>Capillary venous filling time</td>
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<tr>
<td>DFU</td>
<td>Diabetic foot ulcer</td>
</tr>
<tr>
<td>DM</td>
<td>Diabetes mellitus</td>
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<tr>
<td>GTN</td>
<td>Glyceryl trinitrate</td>
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<tr>
<td>HbA1c</td>
<td>Glycated haemoglobin</td>
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<td>HbTBI</td>
<td>Higher baseline TBI—i.e., this foot that had the higher toe brachial index (toe blood pressure) at baseline. Apart from two exceptions, this foot was not exposed to GTN in either the intervention or control groups.</td>
</tr>
<tr>
<td>IAD</td>
<td>Interarm difference</td>
</tr>
<tr>
<td>ID</td>
<td>Interdigital</td>
</tr>
<tr>
<td>ITT</td>
<td>Intention to treat</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<tr>
<td>LbTBI</td>
<td>Lower baseline TBI—i.e., the foot that had the lower toe brachial index (toe blood pressure) at baseline. This foot received the patch (GTN and placebo patches).</td>
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<tr>
<td>LOPS</td>
<td>Loss of protective sensation</td>
</tr>
<tr>
<td>MCI</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>NNT</td>
<td>Number needed to treat</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>PAD</td>
<td>Peripheral arterial disease</td>
</tr>
<tr>
<td>PDN</td>
<td>Painful diabetic neuropathy</td>
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<tr>
<td>PG</td>
<td>Plethysmographic</td>
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<td>PIP</td>
<td>Proximal interphalangeal</td>
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<td>PPG</td>
<td>Photoplethysmography</td>
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<td>PVD</td>
<td>Peripheral vascular disease</td>
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<tr>
<td>RA</td>
<td>Rheumatoid arthritis</td>
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<tr>
<td>RCT</td>
<td>Randomised controlled trial</td>
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<tr>
<td>TP</td>
<td>Toe pressure</td>
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<td>TBI</td>
<td>Toe brachial index, defined as the systolic toe pressure divided by the systolic brachial blood pressure—synonymous with TBPI</td>
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<td>TBPI</td>
<td>Toe brachial pressure index—synonymous with TBI</td>
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<td>Type I error</td>
<td>Type I errors occur if researchers regard their findings as significant when those findings are due to chance (rejection of a true null hypothesis).</td>
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<tr>
<td>Type II error</td>
<td>Type II errors occur if researchers regard their findings as not significant when the opposite conclusion should have been drawn (i.e., the null hypothesis should have been rejected).</td>
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<tr>
<td>VAPS</td>
<td>Visual analogue pain scale</td>
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INTRODUCTION

1.1 The context for the study

Impaired vascular circulation of the feet is a common problem worldwide, associated with advanced age, smoking history, and diabetes. As people age, the incidence of peripheral arterial disease (PAD) rises. The vascular supply to the foot is an important marker for PAD and cardiovascular health (Menz, 2010) and has close associations with cardiovascular disease (CVD; Hyun et al., 2014; Stoenbroek, Ubbink, Reekers, & Koelmay, 2014). CVD is the leading cause of death in the developed world (World Health Organization, 2013). PAD reduces life expectancy by 10 years (de Berrazueta et al., 2003). The risk of mortality in people with PAD is similar to that of people with a history of a previous cardiovascular or cerebrovascular event (Caro, Migliaccio-Walle, Ishak & Proskorovsky, 2005, Mazimba, 2010).

Prevalence of PAD is widely unknown but reports range from 4.3% to 57% depending on the population and how it is assessed (Caro et al., 2005). It can be assumed that PAD is of growing prevalence worldwide due to increases in rates of diabetes mellitus (DM) and ageing populations (National Health and Medical Research Council [NHMRC], 2011; Rogers, Lavery, & Armstrong, 2008; Rooke et al., 2011).

The toe brachial index is the systolic toe pressure divided by the systolic brachial pressure. Abnormal toe pressure is a TBI of < 0.71 (Høyer, Sanderman, & Petersen, 2013b) and is associated with PAD.

A relationship between TBIs and CVD mortality is described by Hyun et al. (2014) who report strong linear associations with these elements in people with and without diabetes. Høyer et al. (2013b), in a review of toe pressure measurement for the diagnosis
of arterial disease, also refer to links between toe pressures and cardiovascular disease but state that this research is at a preliminary stage. TBIs have been associated with known risk factors for CVD such as increased waist measurement and hemorrhheological abnormalities of metabolic syndrome (Gyawali, Richards, Tinley, & Nwose, 2014). Links between cardiovascular risk status and TBIs have been demonstrated with a strong inverse association (p < .001) between the Australian Cardiovascular Risk Calculator and TBIs (Cook, Robinson, & McDonald, 2013).

Stoenbroek et al. (2014) state that early detection of PAD is crucial, not only to provide symptomatic patients with adequate therapy, but also to trigger appropriate intervention for associated high risks of cardiovascular morbidity and mortality. However, only 50% of cases of PAD have any presenting symptomatology (Norgren et al., 2007). This phenomenon contributes to high levels of undiagnosed disease. Clinical signs associated with pulses, temperature, and skin colour and condition, heavily relied upon for screening for arterial disease, have low sensitivity and specificity for the diagnosis of PAD (Williams, Harding, & Price, 2005) and are therefore unreliable. Prior to the recent availability of portable automated photoplethysmographic (PPG) devices for the measurement of TBIs, only laboratory technology could reliably detect and quantify PAD before it was severe.

PAD and other disorders of the cardiovascular system are a major complication of diabetes, responsible for most deaths of people with diabetes at increased rates compared with people who do not have diabetes (National Cardiovascular Disease Prevention Alliance, 2012). There is a worldwide pandemic of diabetes associated with modern diets and sedentary lifestyles that has become an exponentially growing problem over the last century, affecting people in both developed and developing countries (Shaw, Sicree, & Zimmet, 2010). Diabetes causes foot disease from multifactorial impairments, including disease of the vascular and neurological systems. One of the major risk factors for foot amputation is lower limb ischaemia, which is the primary focus of this study within the broader topic of PAD.

Lower limb ulcerations and amputations in diabetes are precipitated by multiple risk factors risks that include changes to the vascular supply in the foot. Amputations
involve high personal, social, and health system costs (American Diabetes Association, 2008; Rogers et al., 2008). The changes in circulation associated with diabetes include low vascular supply pressures, calcified supply vessels, and a reduction in the vascular endothelial responses, all of which are linked to impaired immune responses and reduced function in several systems. These factors contribute to the risks of foot ulceration, infection, and amputation.

The complications, including foot disease, associated with diabetes are poised to overwhelm the resources of health systems on a global scale as the incidence of diabetes rises at an unprecedented rate, outstripping earlier predictions (Shaw et al., 2010). The health care burden is highlighted by Rogers et al. (2008), who state that $21.8 billion US of the costs associated with foot disease could be saved by actuating amputation prevention strategies.

In diabetic foot ulceration, predictors of healing differ between patients with and without PAD. A negative impact of infection on diabetic foot ulcer healing was noted to be confined only to patients with PAD in a study of the most relevant clinical characteristics in these cases (Prompers et al., 2008).

Healing of ischaemic ulceration in cases of PAD is characteristically slow, even when all relevant modifiable factors are optimised (Prompers et al., 2008). According to a meta-analysis regarding diabetic ulcer healing interventions, only 31% of diabetic foot ulcers (DFUs) healed in 20 weeks, with the rest remaining unhealed (Petrova & Edmonds, 2006). A more recent study piloting care with an interprofessional team for DFUs demonstrated a 7-week mean healing time for 69% of cases (Ogrin, Houghton, & Thompson, 2013).

Non-diabetic peripheral ischaemia is also prevalent and growing with the increase in age of populations and as the result of atheromatous disease. Ischaemic disease of the periphery is associated with metabolic disorder and, in particular, smoking history (Rooke et al., 2011).
1.2 The study concept

In the research reported in this thesis, the potential of a pharmacological intervention to improve vascular supply to the toes using glyceryl trinitrate (GTN), an established transdermal patch medication with known vasodilatory properties was explored. Long-acting, slow release transdermal GTN is the mainstay of angina management because of the reliability with which it reduces cardiac preload and afterload from both local and systemic vasodilation. GTN has been used in medicine since the discovery of its bioactive properties 140 years ago.

As a known vasodilator, GTN has the potential, when applied in appropriate doses and in pedal locations, to improve pedal perfusion. Exploration of the existing body of research has unearthed literature that suggests value of GTN in healing and wound prophylaxis for the “at-risk” foot. In addition to the expected well-known vasodilatory effect, outcomes including enhanced wound healing, analgesia in both diabetic neuropathy and ischaemic cases, and even an association with antimicrobial action, are reported. The positive effects reported in the literature justify a thorough investigation of the potential value of GTN for therapeutic applications.

Extrapolating from the body of related literature, it appears that GTN may have the potential to act as both an agent of prophylaxis for foot ulceration, and as a pharmacological adjunct to healing. Given its reported properties of both vasodilation and analgesia, and its documented actions as an inflammatory mediator in wound healing, GTN has value as a potential pharmacological adjunct to standard wound-healing treatment interventions.

The substantial increase of vascular flow, and therefore nutrient delivery, that results from even the smallest increases in vessel lumen, should follow in accordance with Poiseuille’s law\(^1\), given the reliable vasodilatory effect of GTN.

---

\(^1\) Poiseuille’s law

The principle that the volume of a homogeneous fluid passing per unit time through a capillary tube is directly proportional to the pressure difference between its ends and to the fourth power of its internal radius, and inversely proportional to its length and to the viscosity of the fluid.
The main component of this study is an experiment using several measures of both vascular perfusion and neurological function, which was designed to test the effects of GTN in vascularly impaired feet. This experiment was conducted with 100 people, each with initial TBIs below the threshold for the diagnosis of PAD. All of these people were reassessed monthly over 5 months. There were four groups within the study. Two groups received active GTN patches of two different doses. A placebo patch group and a control group provided the contrasts of nonintervention.

The participants in the study were considered representational of the population with “at risk” feet, due to their sub normal toe pressures. The “high risk foot” or “at risk foot” are terms conventionally used when a person has medical, and possibly other, risk factors associated with the potential for foot morbidity. These risks factors are typically understood to include the propensity for tissue damage, the prospect of delayed or incomplete healing from wounds, the increased likelihood of wound recurrence, greater chances of infection and the potential for structural or biomechanical stress and potential deformity. The risk categories are classically include diabetes, reduced vascular supply, impaired neurological function, particularly loss of protective sensation (LOPS), structural and functional deformities, dermatological conditions and self-care including health literacy issues. The person’s level of self care ability, tied in with their understanding and management of their disease process, including being able to reach, see and care for their own feet, or access appropriate care, are each elements that contribute to the high risk foot algorithm.

1.3 Measurement of outcomes

Neurological measures were originally included in the study with the aim of capturing relevant additional information during the same data collection episodes. However the focus of this enquiry was on the vascular outcomes as the primary measure of a treatment effect for GTN on high risk feet.

The ankle brachial index is a commonly used measure of lower limb blood supply, and is obtained by dividing the ankle systolic pressure by the brachial systolic pressure.
When diabetes has been present long enough to cause vascular complications, standard ankle brachial index (ABI) clinical measurements become unreliable due to incompressibility of vessels that results from the calcification that accompanies chronic disease (Hyun et al., 2014). Furthermore, if people have renal failure or advanced age-related vascular changes, their ABIs are likely to be unreliable. Sensitivity of the ABI for detecting PAD drops to only 15% in these high risk groups (Xu et al., 2010).

The ankle brachial index (ABI) has wide application. It is supported by a large body of evidence, providing a valuable screening tool that serves as a marker for atherosclerosis and a predictor of CVD and of all-cause mortality in the general population. In a review of sensitivity and specificity of the ankle—brachial index to diagnose peripheral artery disease (Xu et al, 2008), levels of sensitivity of the ABI ranged from 15–79%. Sensitivity was low, especially in elderly individuals and patients with diabetes. Wikstrom, Hansen, Johansson, Lind, & Ahlstrom. (2008) found very low sensitivity of only 15% for detection of peripheral arterial disease (PAD) in subjects aged 70 years, and concluded that ABI underestimates the prevalence of PAD in the elderly.

In a review article by Høyer, Sandermann & Petersen (2013b), the toe brachial index (TBI) had a sensitivity of 90% to 100% for PAD detection. These data came chiefly from populations being screened for vascular disease although some normal populations were included. Toe pressures remain a sensitive test for PAD in the elderly and in diabetes, both conditions that place feet at the highest risk of ulceration. Toe pressures are recommended in international guidelines when ABIs are elevated or inconclusive. Therefore, in this study TBIs were used in place of ankle brachial indices to overcome the lack of sensitivity in the population of interest. Toe pressures are widely recommended by national and international guidelines in assessment of peripheral vascular supply (Bakker, Apelqvist, & Schaper, 2012; Colaguri et al., 2013; NHMRC, 2011; Norgren et al., 2007; Rooke et al., 2011), as a useful adjunct in vascular assessment for high risk populations when other assessments have become unreliable.
due to hardened vessels. See Appendix B for a table of these guidelines and their specific recommendations regarding the use of toe pressures in vascular assessment.

This study uses a novel version of PPG technology for clinical vascular assessment. When seeking an appropriate method to measure changes in vascular supply to the most distal part of the foot, the availability of new technology in the form of a reliable, validated, portable device for the measurement of toe perfusion came to light. The first three of these automated toe pressure units were imported to Australia for this study. Measurement accuracy was maximised by adherence to specific test protocol and conditions.

1.4 The gap addressed by this research

Reversing the paucity of research in podiatric applications of GTN is essential for exploring and exploiting any potential of this pharmacological therapy. This project addresses this need and aims to contribute a clinically relevant understanding of the use of GTN in cases of impaired pedal vascular supply.

This research addresses a gap in the methods available to treat foot ulceration as recommended by Petrova and Edmonds (2006) in their review of emerging drugs for diabetic foot ulcers. They state that angiogenic stimulants and nitric oxide donors should be trialed when diabetic foot ulcers fail to heal. GTN has both of these properties.

GTN is not currently used in the standard treatment of high-risk patients for podiatric conditions. Given its reported properties of vasodilation and analgesia, and its actions as an inflammatory mediator in wound healing, GTN has potential as a pharmacological adjunct to standard wound-healing treatment interventions, having been reported to act as an agent of wound prophylaxis for people at risk of PAD and diabetic foot complications. Its analgesic properties, reported for both ischaemic and neuropathic pain, are of therapeutic interest and clinical value. The potential of these applications for these prevalent and complex conditions is, to date, largely unexplored and untapped. This study seeks to clarify some of the possibilities and practicalities of application of GTN to neurovascular foot problems. It provides information that can direct subsequent research.
1.5 Researcher background and motivations

The possibility that GTN could improve vascular perfusion, and thereby benefit people at risk of foot ulcers and the associated health risks, was the impetus for this doctoral project. As a podiatric clinician treating high-risk clients with foot ulcers, this topic held considerable professional interest for me. I saw this enquiry as a way of making a unique, novel, and potentially powerful contribution to the body of knowledge in an area where the health and quality of life of people with PAD might be directly enhanced. The implications for benefits to worldwide health systems are apparent.

In addition to being a podiatric clinician, I am a clinical educator. I therefore carry the responsibility of disseminating the most appropriate and evidence-based clinical solutions to the health practitioners of the future, and to inform practice more widely with the university’s research output. As well as the primary research findings regarding toe pressure and GTN use, this enquiry provided evidence about the validity of neurovascular assessments as historically taught to podiatry students. Some of these findings should trigger changes of practice.

1.6 Thesis structure

This thesis is divided into six chapters. Following this introductory chapter, Chapter 2 contains a set of case studies that acted as the stimulus for the research reported in this thesis. In these case studies, GTN patches were used on four clients presenting with foot ulcers at the Charles Sturt University (CSU) podiatry clinic. Each of these cases had advanced PAD and demonstrated rapid healing of ulceration. Together, they provided a compelling impetus to research the apparent therapeutic effects of GTN.

Chapter 3 comprises a literature review in three sections. It commences with a background of PAD, then covers the wide-ranging applications of GTN that harness its reliable vasodilatory properties. The value of GTN is documented, commencing with the literature from 140 years ago when vasodilation provided breakthrough therapy for angina management, a use for which GTN remains the treatment of choice. Literature describing GTN’s wound healing effects in both human
and animal studies is presented. Following a consideration of GTN’s current uses, this chapter proceeds to focus on its potential in therapeutic applications for the foot. Considerations associated with the use of GTN are detailed including its highly variable absorption, tolerance, and local versus systemic effects. The necessary considerations relating to contraindications, interactions, precautions, and side effects are also given here.

The final section in the literature review deals with the assessment items and tests used in both the pilot and the main experiment that are the primary focus of this project. A number of methodological considerations and issues are discussed, and explanations are provided that justify the methods selected. Issues associated with the reliability and validity of clinical vascular, neurological, and wound assessments are addressed in preparation for the pilot study and the main experiment.

In Chapter 4, the pilot study for the main experiment, is described. This proved to be invaluable for informing the choices of assessment items and protocol for the subsequent experiment, and additionally provided insights applicable to both the teaching and practice of podiatry.

Chapter 5 contains the experiment that forms the main empirical component of the research reported in this thesis. It commences with an introduction in which there is a description of the overall design of the study, followed by information that indicates the need for exploring the nature of TBIs. The rationale for the GTN dose selections, and the research questions and hypotheses are also presented. This is followed by a description of the methods used, the results, and an experiment-specific discussion. The results commence with a comprehensive description of the participants, then the TBI data are presented and analysed in a number of ways that include refining them and exploring their characteristics. The effects of GTN on TBIs are then evaluated using ANCOVAs as the main research output, and finally, analyses of responders versus nonresponders to GTN are presented.

Although chapter-specific discussions are provided at the end of Chapters 2, 4, and 5, Chapter 6 comprises a discussion and set of conclusions that relate to the thesis as a
whole. In this final chapter, the outcomes of this work are integrated along with consideration of the implications for clinical podiatric education and practice. The clinical relevance of findings regarding toe pressure measurement is highlighted, followed by a consideration of the effects of GTN generated from the case studies (Chapter 2) and the experiment (Chapter 5). Within this final chapter, a vascular assessment pathway is proposed that includes medical referral for the management of cardiovascular risk in the presence of PAD. Recommendations are also made for the clinical application of GTN, providing guidelines so that clinicians and their patients can work together to determine individually effective doses. Throughout this final chapter, reference is made to the areas that hold potential for future research.
FOUR CASE STUDIES

2.1 Introduction

My interest in the use of GTN as an adjunct to wound healing was triggered when the initial case described in this chapter presented to the university podiatry clinic and his toe ulcer proceeded to deteriorate during the first 3 weeks of evidence-based and holistic wound care. This client manifested a set of risk factors that suggested an increasingly poor outcome with complications involving morbidity and even mortality.\(^1\) When his TP was measured at just under the threshold of normal, there was a clear need to boost his vascular supply.

The mention of GTN at a previous wound care conference presentation came to mind. A preliminary search of Medline identified six articles about the use of GTN in feet. Five of these pertained to veterinary applications of GTN to horses’ hooves, and the single reference to human podiatric applications reported an insignificant result (Kim, Ballinger, & Kushner, 2006).\(^2\) I commenced further searches of the medical literature pertaining to GTN in both human and animal use, the results of which are presented in the following chapter.

As a result of my initial exploration of the literature, the case mentioned above and three additional cases of chronic toe ulceration were treated with low doses of topical GTN. They proceeded to rapid healing after the addition of this agent to their wound care regimens. These cases consisted of two non-diabetic ischaemic males and two diabetic ischaemic males who were treated as foot ulcer clients consecutively presenting to the podiatry service in the Allied Health Clinic at Charles Sturt University, Albury in 2010. In each case, evidence-based best practice wound care was used with monitoring at least weekly, and

\(^1\) This man’s wife of 70 years had recently died. Death rates increase by 66% in the first 3 months after loss of spouse due to “the widowhood effect” (Moon, Glymour, Vable, Liu, & Subramanian, 2013).

\(^2\) Information about this article is provided in the literature review in Chapter 3.
transdermal GTN patch treatment was added with the aim of increasing vascular perfusion to the ulcerated extremity to facilitate wound healing. GTN was procured through requests for prescriptions from the patients’ GPs, with 5 mg, the lowest commercially available dose. Each of these cases demonstrated rapid and uninterrupted progress to healing as well as improvements in toe pressures (TPs) according to readings from a manual single-cuff photoplethysmography (PPG) device. Clinical assessment, management, and outcomes for each case follow.

2.2 The four cases

2.2.1 Case 1

2.2.1.1 Presentation

A 93-year-old man who had been an existing client of the university podiatry service presented in midwinter with ulceration and pain that had been present for approximately 2 weeks at the apex of his left hallux. Significant medical history included PAD, cardiac failure, hypertension, chronic obstructive airways disease (COAD), osteoporosis, and gout. His medications included frusemide, allopurinol, celecoxib, ibopufen, an H$_2$ agonist, a biphosphonate, and an anticholinergic (for COAD). Due to this combination of factors he was a high risk case for both podiatric and medical issues.

Significant social history included the recent loss of his long-term spouse. Since this event, he had lived alone, with daily visits from his family. Podiatric history included ingrown toenails of the halluces, painful subungual onychophosis of the hallux nails, and plantar heloma dura (corns) for which he had been receiving 4 years of episodic podiatric care. On examination, two apical lesions were present on his left hallux. One lesion consisted of a patch of necrotic skin measuring approximately 4 mm x 6 mm. The second lesion consisted of an area of broken epidermis with a bright red dermal base measuring 3 mm x 4 mm. See Figure 2.1. The lesions were suggestive of erythema pernio (chilblains) that had progressed to superficial necrosis and superficial arterial ulceration. The feet were hairless with dusky pallor in the forefoot and sole with distal rubor, particularly of the apices of the toes. These signs are all indicative of arterial insufficiency.
2.2.1.2 Assessment

Classic signs of ischaemia were present, with cold, atrophic skin, dusky rubor of toes when dependent, reduced toe pulp, absent hair, absent peripheral pulses, and ulceration. A previous Doppler ABI assessment result of 0.75 had demonstrated reduced perfusion (normal $>$ 0.8). The necrotic area was fluctuant and painful when palpated. Systolic TP measurement was 60 mm Hg at presentation on the affected toe.

No Doppler ABI measurement was procurable at this presentation due to his non-compressible ankle vessels. The subnormal PPG TP reading of 60 mm Hg suggested the presence of peripheral arterial disease and associated ischaemia.\(^3\)

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\(^3\) Note that TPs, not toe brachial indexes (TBIs), were measured for this case and the other three cases in this chapter.
2.2.1.3 Management and outcomes

The patient initially received standard wound care including foam dressings, foam, hydrogel, and fixative tape changed by the carer at 2–3 day intervals. At the first visit, a recommendation was made for sheepskin boot slippers to maintain warm thermal status of the feet. The patient’s nutrition was also assessed. Nutritional advice to maintain adequate levels of protein, vitamin C, and zinc was given to the patient and his carers. The recommended improvements to both his diet and his footwear had been implemented by the following visit, 1 week later.

The initial progress of this man’s wound healing was retrograde, with an enlargement of the broken skin lesion after 20 days of treatment. By then, the apex ulcer had lengthened by 2 mm, the base of the ulcer was pale, and the wound edge appeared to be poorly perfused. There was also a deterioration in colour from that shown in Figure 2.1. See Figure 2.2, and refer to Table 2.1 for progressive assessments.

Figure 2.2. Case 1: Exacerbation of wound at 20 days with standard care.
### Table 2.1

**Case 1. Baseline Characteristics and Subsequent Observations**

<table>
<thead>
<tr>
<th>Timeframe</th>
<th>TP mm Hg</th>
<th>Ulcer characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial presentation</td>
<td>60</td>
<td>3 mm x 4 mm = 12 mm² Red shallow base. Figure 2.1.</td>
</tr>
<tr>
<td>20 days post presentation</td>
<td>–</td>
<td>2 mm x 9 mm = 18 mm² Pale base, pale skin flap with poor perfusion proximal to ulcer. Figure 2.2.</td>
</tr>
<tr>
<td>GTN therapy commenced</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 days of GTN</td>
<td>65</td>
<td>1.5 mm x 8mm = 12 mm² Bright red granulating base, active healing wound edges. Figure 2.3.</td>
</tr>
<tr>
<td>10 days of GTN</td>
<td>–</td>
<td>Epithelialised. No exudate on dressings reported by carer. Reduction in pain.</td>
</tr>
<tr>
<td>21 days of GTN</td>
<td>70</td>
<td>New lesion in nail sulcus, but apex remained healed. Figure 2.4.</td>
</tr>
<tr>
<td>After 4 weeks of GTN</td>
<td>70</td>
<td>Apex healing consolidated; sulcus healed. Figures 2.5 and 2.6.</td>
</tr>
</tbody>
</table>

At 20 days, a decision was made to commence unilateral 5 mg GTN patch therapy to the left foot with the aim of improving vascular supply to this extremity. A further increase in vitamin C intake was recommended at this visit. TP and wound progress were monitored at subsequent clinic visits.
The lesion was reviewed 6 days after initial GTN patch application. Reduced wound size, advancing edges, and granulating base of good colour were evident, indicating that wound healing was in progress. Toe pressure measurement had increased by 5 mm Hg to 65 mm Hg at this review. See Figure 2.3.

![Figure 2.3](image)

*Figure 2.3. Case 1: Progression of healing after 6 days of GTN therapy. Note improvements in wound base colour and vitality of wound edges.*

The primary carer was asked to note on which day there was no further exudate present on the dressing he was changing daily. This occurred on Day 10 according to his report.

The apical ulceration had healed at the next review at 21 days of GTN treatment. See Figure 2.4. TP had improved further to 70 mm Hg. There was, however, a new wound in the medial nail sulcus at a site of previous onychophosis and onychocryptosis. This lesion was due to a combination of tissue fragility and ingrowing nail pressure.
At Week 4 of patch therapy, healing was complete in both previously broken skin areas. The necrotic area had reduced in size, was a lighter colour, and was no longer soft or fluctuant. See Figures 2.5 and 2.6. TP remained at 70 mm Hg. The presenting pain was largely resolved. The now resorbing necrotic area was still painful, but only when palpated.
2.2.2 Case 2

2.2.2.1 Presentation

This case, a 93-year-old non diabetic male, presented with a painful, ulcerated left third toe of about 2 weeks duration. His medical history included severe PAD and a below-knee amputation of the right leg due to ischaemic digital gangrene 7 years previously. He had previous surgical repair of an aortic aneurism and had been a smoker until 10 years prior to his initial presentation to the clinic. His medications were aspirin, an ACE inhibitor, a statin, and an antidepressant. He was living in an assisted care situation in an aged-care hostel. He presented with a member of staff. He was not ambulatory for more than a few meters, but could transfer from his wheelchair to the treatment chair. He wore a lower leg prosthesis.

The ulcer was located on the proximal interphalangeal (PIP) joint of the third toe. The third and fourth toes were impinging at the PIP joints, the pressure contributing to the etiology of the ulcer, resulting in the interdigital ulcer of about 2 mm diameter. The ulcer had features characterising arterial insufficiency: a punched out, circular lesion with a central yellow, sloughy base. The patient complained of significant unremitting pain in his left third toe, worse at night and interfering with sleep.

2.2.2.2 Assessment

His lower limb was cold, cyanosed, and hairless, with atrophic skin. The pulses were not palpable, nor were they audible to Doppler examination, so no ABI was procurable. TP measurement was 38 mm Hg, indicating significantly impaired perfusion.

2.2.2.3 Management and outcomes

The lesion was dressed with standard hydrogel and a silicone dressing. A 2 mm compressed adhesive felt spacer with a deflective cutout for the lesion and its dressing was applied between the toe apices to reduce the interdigital impingement pressure at the PIP joint. The first 5 mg GTN patch was applied on the same afternoon of the initial presentation. When reviewed 3 days later for his first dressing change, the ulcer had completely resolved. TP
had improved from 38 to 42 mm Hg. He reported he had experienced significant pain relief and had been able to sleep relatively unaffected by night pain. A new bruised area associated with the pressure of the felt spacer on the fourth toe was noted.

The client was monitored twice weekly over the next 17 days. TPs increased to 58 mm Hg by the 14th day of the GTN patch treatment. See Table 2.2 for documentation of the changes in TPs over this time. The client and carers noted some rubor over the site of application on the dorsum of the foot. This did not cause discomfort to the patient and was successfully managed by varying the site of each new patch application. The interdigital (ID) bruise resolved within 10 days of its initial appearance.

Table 2.2

Case 2. Baseline Characteristics and Subsequent Observations

<table>
<thead>
<tr>
<th>Timeframe (days)</th>
<th>TP in mmHg</th>
<th>Ulcer characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 GTN commenced</td>
<td>38</td>
<td>ID ulcer, 2 mm diameter x 1mm deep sloughy base, painful</td>
</tr>
<tr>
<td>3 days of GTN</td>
<td>42</td>
<td>Ulcer healed, ID bruise noted</td>
</tr>
<tr>
<td>7 days of GTN</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>10 days of GTN</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>14 days of GTN</td>
<td>58</td>
<td>Bruise resolved</td>
</tr>
<tr>
<td>17 days of GTN</td>
<td>42</td>
<td>Ulcer remained healed</td>
</tr>
</tbody>
</table>

The drop in TP at Day 17 raised the issue of the possible development of gradual drug tolerance. This is known to occur and lead to failure of effect as biochemical reactants in the metabolism of GTN become exhausted with continuous use (see Chapter 3, Section 3.4.6 for details). This phenomenon is managed in standard applications of GTN (where it is used as a mainstay of angina management) by 8–12 hour rest periods during every 24 hours of application. This phenomenon is dose dependent, and small doses are reportedly not subject to tolerance (Uxa et al., 2010).

2.2.3 Case 3

2.2.3.1 Presentation
The third case, an 83-year-old man, had been a client of the CSU Allied Health clinic podiatry service for the previous 12 months due to chronic apical ulcerations on both halluces and venous leg ulcers of several years duration. His medical history included complex venous disease, chronic weeping cellulitis, severely ulcerated legs (at times the ulcers were circumferentially continuous around his lower legs), and a 13-year history of diabetes. He was an adept and consistent user of compression stockings. His medications included simvastatin, an angiotensin II receptor antagonist, a calcium channel blocker, an ACE inhibitor, an H₂ receptor agonist, vitamin D, metformin, ivabradine, a biphosphonate, glycazide, and antibiotics episodically for his leg ulcers. He was still actively working on his own farm. He complained of pain in both feet that was waking him at night.

His hallux ulcerations had been present for 6 years on the right toe and 3 years on the left. See Figure 2.7.

Figure 2.7. Case 3: Long-term apical ulcerations of both halluces.

2.2.3.2 Assessment
Stable TPs measured at 90 mm Hg suggested elevated pressure readings due to incompressible vessel calcification associated with his long-standing diabetes and PAD. Over the previous year, he had received standard treatment from the university clinic, including weekly wound care, offloading insoles, bespoke boots, and vascular specialist management. During this time, his apical ulcerations persisted unhealed, and he continued to have episodic cellulitis with multiple areas of ulceration on both legs. It was unclear whether the primary etiology of his nocturnal leg pain was arterial rest pain, venous incompetence, diabetic neuropathic pain, or a combination of these. However, he gained some relief from sleeping with his feet in dependency, which indicated an arterial deficiency component.

2.2.3.3 Management and outcomes

Initially, a 5 mg GTN patch was prescribed for this man’s right foot only, with the intention that his untreated foot would act as a control to determine any local effects. When he came in for review 1 week later, he had been using the patches on both feet due to the significant relief experienced from his nocturnal foot pain, which was reduced from 10/10 to 7/10 on a 10 point visual analogue pain scale (VAPS). At this time the right hallux had a more vascular presentation with fresh bleeding present. See Figure 2.8. This was in contrast to the indolent, pale, and dry appearance that had previously characterised his toe wounds.

At 3 weeks post GTN the right ulcer was much reduced in size and the surrounding tissue was normalising. See Figure 2.9. In addition, his left hallux ulcer of 3 years had healed.
Figure 2.8. Case 3: Right hallux after 1 week of GTN application. Note decreased wound size and increased vascularity.

Figure 2.9. Case 3: Right hallux improving at 3 weeks post GTN.

A loose piece of bone extruded from the apex of his right hallux during debridement of hyperkeratosis at 4 weeks post patch application. The bone fragment suggested underlying osteomyelitis, which was confirmed by X-rays and pathology testing. Vascular specialist opinion was sought at this time, but surgery was considered to be contraindicated due to his complicated medical history. He continued to use the patches, and within another 3 weeks the right hallux ulcer of 6 years had also healed. See Figure 2.10. Note the markedly improved skin condition. Figure 2.11 shows both toes healed. Patch therapy was then discontinued by the patient of his own volition. He had felt it was “no longer assisting him”.
Both ulcers recurred within a few weeks of ceasing the GTN patch therapy. See Figure 2.12 for a typical view of incipient reulceration of the right hallux. Glyceryl trinitrate treatment was then reinstated. This time, a daytime rest period from the GTN of 8 to 12 hours to avoid drug tolerance was recommended, with the patches to be worn through the night. The right foot healed again within 1 week and the left foot improved each week and had healed again within 4 weeks.
Figure 2.12. Case 3: Recurrence of ulceration after ceasing GTN.

This patient (Case 2) went through a further three repeat cycles of breakdown when GTN was discontinued, and subsequent rehealing of the toe ulcers occurred each time within a few weeks following resumption of GTN patch therapy. When he was directly questioned about why he kept ceasing the GTN treatment, he stated that he felt that the GTN was “making his legs break out”. Typical leg wounds for this man during that time are shown in Figure 12.3. His assertion was considered valid, as GTN could conceivably worsen venous ulceration by the same vasodilatory mechanism proposed to improve arterial ulceration. This could occur because of vasodilation and the subsequent increase in vascular pressure. The etiology of venous leg ulceration involves increased interstitial fluid and edema, with incompetent venous return and resultant stasis creating a predisposition to tissue breakdown. Glyceryl trinitrate use was subsequently restricted in his case to prophylactic application only for incipient apical toe wounds of arterial etiology whenever they appeared imminent.

A management plan using episodic transdermal GTN was aimed at maintenance of the healed status of his toe ulcers and minimisation of any detrimental effects on his chronic venous leg wounds from GTN’s vasodilatory effect. Figure 2.14 shows his legs at their best overall condition achieved, with minimal breakdowns visible as his needs for both toe and leg ulcer control were balanced with ongoing short-term episodic GTN treatment.
Figure 2.13. Case 3: Example of leg wounds.

Figure 2.14. Case 3: Improved leg skin condition.
2.2.4 Case 4

2.2.4.1 Presentation

Case 4 was a 76-year-old man with long-term history of Charcot Marie Tooth disease, cardiomypathy, hypertension, obesity, COAD, and Type 2 diabetes of 8 years’ duration. His medications included a beta blocker, glicazide, frusemide, metformin, atorvastatin, and aspirin.

2.2.4.2 Assessment

This man had signs of arterial insufficiency including atrophic skin, painfully cold feet, a history of toe ulcerations and chilblains each winter, and high pressure areas on heels and toes related to the cavus foot deformity associated with his neurological condition.

2.2.4.3 Management and outcomes

Care for this man included management of his apical ulcerations with pressure offloading insoles, custom-made footwear, and a standard wound dressing regimen.

He had a single small but persistent ulcer on the apex of his right second toe. See Figures 2.15 and 2.16. This wound went through several episodes of recurrent infection over 7 months. He was prescribed oral antibiotic medication after topical antibiotic treatments failed. Although the oral antibiotic therapy temporarily cleared the infection, the ulcer had remained unhealed for 7 months.

Figure 2.15. Case 4: Chronic apical ulcer of 7 months duration.

Charcot Marie Tooth disease is a neurological disorder with characteristic loss of distal plantarflexory muscle function.
Four days after transdermal GTN was commenced, in conjunction with antibiotics, this wound healed. See Figure 2.17. Note the improved appearance of the skin condition compared with the previous two photos.

The wound broke down but rehealed 7 days later, 11 days subsequent to the commencement of GTN, after which time healing consolidated. His 5 mg GTN dose was later split to 2.5 mg on each foot with the aim of providing better long-term stability and extended freedom from ulceration on both feet.

2.3 Discussion

Case studies of the kind reported above can be regarded as having intrinsic limitations in terms of bias in selection of the participants studied, lack of generalisability to wider
populations, and an inability to categorically establish cause and effect. However, because these four ulcer cases represent consecutive presentations at the university clinic at that time, they are less subject to bias than specially selected cases. Nevertheless, whether the outcomes achieved with these cases could be generalised to other people who have foot wounds and peripheral artery disease, and indeed whether the outcomes could be attributed to GTN, are open issues.

That aside, these four case studies are presented in the context of an exploratory process that revealed some possibilities that were worthy of pursuit, especially given the acute international need for effective agents in the management of lesions of high-risk feet. They shed light on four areas of interest. First, GTN appeared to play a role in promoting very rapid ulcer healing in contrast to the average healing time for a diabetic foot ulcer of 20 weeks (Petrova & Edmonds, 2006).

Second, toe pressures increased with the application of GTN in each case. In Case 1, a 10 mm Hg (16%) increase from baseline occurred. In Case 2, there was a larger increase of 20 mm Hg (53%), which was remarkable due to this man’s advanced age (93 years) and significantly pathological vascular status (lower limb amputee). Toe pressures in Case 3 were very stable and high at 90 mm Hg, suggesting that arterial calcification was present. Toe pressures in Case 4 were unfortunately not measured before commencement of GTN therapy. However, a change from 40 mm Hg at Day 5 to 80 mm Hg at Day 10 (a 100% increase) occurred on his right foot.

Third, toe pressures are difficult to measure accurately due to the large degree of inherent error. This area was identified as requiring controlled research. The measurements of pedal perfusion referred to in these case studies are limited to TPs as distinct from toe brachial indices (TBIs) which incorporate the systemic blood pressure (BP) and may give more valid measure of relative vascular perfusion.

Fourth, GTN at the dosages used for these four men, did not appear to have a generalised effect. All of them were questioned about side effects. None of them initially identified
headaches\textsuperscript{5} or other side effects, and none developed any significant skin reaction in the short term. Slight skin rubor occurred at the patch contact site for Cases 1 and 3, after which the patch site was strictly rotated around the dorsum of the foot. This resulted in resolution of skin changes within a week. Case 3 eventually associated some minor exacerbation of his pre-existing leg ulceration with the use of GTN. However, his leg skin condition remained far better overall than at initial presentation.

While three of these cases were already being treated at the university clinic for unhealing ulcers, Case 2’s ulcer was an early presentation that might have healed without GTN. However, the rapidity of healing and pain relief in this case with such advanced comorbidities was remarkable.

Case 3 provided an opportunity to observe in at least 3 clear cycles, the repeated healing effect as he withdrew then recommenced GTN patches, reulcerating at the same sites after withdrawal and rehealing with reinstatement of the therapy.

Peripheral arterial disease (PAD) was an underlying condition in each of these cases. It could be hypothesised that GTN had some positive effect on amelioration of the affects of PAD, by improving the arterial supply. The association with GTN and healing has been postulated from these case studies but further research on the validity of this association is needed before definitive conclusions can be drawn regarding a GTN effect on foot ulcers.

2.4 Outcomes

These four cases indicated that GTN patch therapy may be a useful addition to the range of therapeutic options in chronic wound care. The outcomes suggested value in exploring the potential of GTN for reducing pedal ischaemia and in the most appropriate ways to conduct preliminary research. In particular, the improvements in toe pressures invited investigation with more accurate and validated toe pressure measurement methods. Determining the ideal dose(s) and regimen of GTN, and suitable conditions for its application, presented a range of methodological questions. These, together with the possibility that GTN could be

\textsuperscript{5} The most common side effect known to occur with GTN is headache, associated with generalised vasodilation. Information concerning this is provided in subsequent chapters.
used to reduce pedal ischaemia, were addressed initially with a review of the literature (reported in the next chapter), then with an examination of relevant methodological issues and a pilot study (reported in Chapter 4), and finally through the experiment that is the major focus of this thesis (reported in Chapter 5).

2.5 Postscript

Case 4 was followed up during, and after completion of, the experimental work reported in this thesis. He had continued with GTN, reporting benefits of more comfortable and warmer feet, with ongoing relief from ulceration. His dose had been titrated down further to 1.25 mg for each foot, which provided the same symptomatic relief and was less subject to tolerance. After 18 months of GTN use, he drew attention to some telangiectasia (spider veins) on his feet that he believed to be new. Although these were initially minor and appeared to be of a degree typical in ageing skin, photographs were taken. See Figures 2.18 and 2.19.

This man continued to use GTN patch therapy consistently for an additional 2 years with the lower dose of 1.25 mg on each foot for 12 hours each night. He remained essentially ulcer free, notably throughout winter. After 3.5 years of GTN therapy, both his feet were
florid with asymptomatic telangiectasia over their dorsal surfaces. This was more marked on his right foot. See Figures 2.20–2.23.

*Figure 2.20.* Case 4: Both feet with marked telangiectasia after 3.5 years of GTN.

*Figure 2.21.* Case 4: Marked telangiectasia on right foot after 3.5 years of GTN.

*Figure 2.22.* Case 4: Right foot lateral side with marked telangiectasia after 3.5 years of GTN.

*Figure 2.23.* Case 4: Left foot dorsum with marked telangiectasia after 3.5 years of GTN.

Telangiectasia are usually associated with venous insufficiency in conjunction with a cluster of signs and symptoms, most commonly oedema; varicosities of varying sizes; haemosiderin deposits, typically in the gaiter area; and venous eczema. Because none of
these other signs of venous insufficiency were present, the appearance of telangiectasia is probably due to angiogenesis. This observation confirmed the value of having explored the effect of GTN in podiatric applications not only theoretically but also empirically via experimentation.
LITERATURE REVIEW

3.1 Introduction

Impaired vascular supply to the foot is associated with an international diabetes pandemic along with CVD and PAD of ageing populations. Efficacy of GTN as an adjunct in treating foot ulcerations was suggested by the results of the four case studies in the previous chapter. Therefore the literature review reported in this chapter was undertaken, seeking evidence of the effectiveness of GTN as a vasodilator to the extremities.

3.1.1 Chapter structure

This literature review is in three sections, commencing with background about PAD relevant to this project. This is followed by section 2, regarding GTN, providing an overview of the broad scope of GTN applications before turning the focus on potential applications of GTN for feet with compromised vascular supply. It commences with the earliest literature about the discovery of the bioactive properties of GTN, and a description of its development as a pharmacological agent. The pharmacology of GTN is addressed, with information about absorption, dose issues, and the related phenomenon of tolerance. The existing scope of applications of GTN is outlined, including uses distinct from its best-known applications for angina pectoris. The subsequent focus is on GTN for use on the extremities and in wound healing. In addition to its best known property of vasodilation, some other properties relevant to wound repair are outlined. The third section of this review covers the literature pertinent to the assessment items proposed for the trial of GTN on people with PAD.

3.2. Peripheral artery disease

3.2.1 Physiology
The human vascular tree is perfused with blood in accordance with the physical laws of haemodynamics. Poiseuille’s law (see foot note1) describes the relationships of flow velocity and pressure to the radius of a vessel. Because of the very powerful influence on resultant volume of the vessel radius (raised to the power of 4), any narrowing of a vessel has a highly significant influence on the resultant vascular perfusion. These effects of arterial stenoses of PAD therefore impair hemodynamics, leading to major reductions in distal blood flow volumes and pressures. These impacts are additive when 2 or more occlusive lesions are located sequentially within the same artery (Domingez & Rowe, 2015). This can result in either symptomatic or asymptomatic reductions of vascular supply to tissues, especially in the periphery and, increased risks of cardiovascular disease including myocardial infarction (MI) and cerebrovascular accident (CVA).

Large vessels show a predilection for stenoses in some disease patterns. Diabetes and renal disease are known to cause both small and large vessel disease. The ankle vessels are typically affected many years before toe vessels become affected with pathology including stenoses and calcification. Disease in peripheral vessels indicates that other vessels in the more proximal vascular tree are also affected. A distinction is made here between PAD, where both large and small vessels can be permanently affected with atherosclerotic lesions, and the temporary superficial disorder of erythemia pernio (chilblains) that may both manifest with the same appearance, i.e. that of superficial distal tissue damage, which may ulcerate. The former has its etiology based in the ischemia resulting from the underlying arteriosclerosis, while the latter is a temporary, transient and self-limiting condition. Both are initiated by the vasoconstriction and relative ischemia of peripheral blood vessels that is a normal exposure to cold temperatures, and while this is prevalent in people with PAD, it is also seen commonly in people without vessel disease, typically younger women 15-30 but may occur in children and older adults as well (Edgerton 2015).

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1 Poiseuille’s law
The principle that the volume of a homogeneous fluid passing per unit time through a capillary tube is directly proportional to the pressure difference between its ends and to the fourth power of its internal radius, and inversely proportional to its length and to the viscosity of the fluid.
3.2.2 Prevalence of PAD

The prevalence of PAD in the general Australian population is unknown, according to the Australian Institute of Health and Welfare, which states that no data are available for this (AIHW 2015). Other authors who performed a large population study in Canada, with a sample size of 16,440 people with PAD, state that the reported prevalence of PAD spans 4.3% to 57%, depending on the target population and how it’s assessed (Caro et al. 2005). Risk factors for PAD are increasing age, smoking, diabetes and male gender (Rooke et al. 2011). Sedentary lifestyle, hypertension, hyperlipidaemia and family history are also linked to PAD prevalence (Domingez & Rowe, 2015).

Several factors complicate the study of the epidemiology of PAD. The most important of these is the lack of symptomology in the majority of people affected. Studies show that asymptomatic PAD is at least as prevalent as asymptomatic PAD (Hooi et al. 2003). When claudication is used as an indicator, it is estimated that 2% of the U.S. population aged 40-60 years and 6% of the population older than 70 years are affected (Domingez & Rowe, 2015).

Vascular laboratory studies have been necessary for diagnosis of asymptomatic PAD up until the recent advent of clinically accessible diagnostic means of portable toe pressure measuring devices. Hoyer 2013, in his review of PAD diagnosis with TBI provides evidence to show that when PAD is diagnosed with traditional clinical algorithms (screening with pulses and ABI as widely recommended and outlined in the NHMRC guidelines and the American Heart association), a marked underdiagnosis of PAD occurs. This was underdiagnosis was estimated by Høyer, Sandermann & Petersen (2013b) to be around 60% of the people sent for laboratory vascular assessment and found to have PAD, where this remained undetected in standard clinical screening.

The Australian diabetes, obesity, and lifestyle study (Tapp, Shaw, De Courten, Dunstan, Welborn, & Zimmet 2003), comprising a population-based sample of Australian adults aged 25 years or older, sampled 11,427 adults, in 42 randomly selected areas of Australia, looking for risk factors for foot complications in type II diabetes. PVD was defined using an ABI of < 0.9 and the Edinburgh claudication questionnaire.
They found the prevalence of PAD was 13.9% in those with previously diagnosed diabetes and 6.9% in those with newly diagnosed diabetes. The authors concluded that the prevalence of PAD was lower than has been reported, and stated that this may reflect differences in sampling methods between hospital based versus community populations. The use of ABI is questioned as a screening tool for PAD in people with diabetes, due to its reportedly low and variable sensitivity for PAD screening in diabetic populations (Williams 2005, Høyer et al. 2013).

3.2.3 Costs and outcomes of PAD

Because of the lack of clear and accurate epidemiological data on the prevalence of PAD, any estimates on the specific costs of PAD alone are unavailable. However, the culmination of the effects of PAD in outcomes of cardiovascular disease and diabetes have been well reported. Some details of both worldwide and Australian health system costs thus related to PAD are provided in Chapter 1, in the introduction to this thesis. A substantial proportion of people with diabetes (19.6%) were determined to be at risk of foot ulceration from the vascular and neurological risk factors determined from the Australian Diabetes Obesity and Lifestyle study (Tapp et al. 2003). NHMRC guidelines estimated the cost of lower extremity amputations in Australia to be $A 26,700 per amputation (Ray, J.A., et al. 2005).

There is a body of literature on the treatment and outcomes of PAD in symptomatic claudicants, but a relative dearth, until recently on populations with asymptomatic PAD. The particular impact of asymptomatic PAD has been “underecognised, underappreciated and undertreated” (Caro et al. 2005, Criqui 2001, Diehm et al. 2009, Hooi et al. 2003, Lange et al. 2004) but a growing body of evidence shows that PAD is a valuable indicator for previously undiagnosed and modifiable CVD risk (Hooi 2003, Hyun et al.2004).

This is relevant as it has been recently identified that people with asymptomatic PAD are actually at higher risk of morbidity and mortality than those with symptoms (Diehm et al.2009, Hooi 2003, Hyun et al. 2004). It has been postulated that this may be because the symptomatic people are recognised and treated by their attending medical practitioners for the concomitant CVD risks. “At least 25% of coronary patients have
sudden death or nonfatal myocardial infarction without prior symptoms. Therefore, the search for coronary patients with subclinical disease who could potentially benefit from intensive primary prevention efforts is critically important” (Greenland et al. 2001).

Symptomatic ischemic pain may occur at rest or as exertional leg pain, when vessels claudicate with exercise. Many older people accept that limitations on their mobility are to be expected, accepting a decrease in walking distance as a normal part of aging, and it is estimated that 50-90% do not complain of claudication symptoms according to Domingez & Rowe, (2015). Diagnosis is also further complicated when comorbidities are present. Symptoms from osteoarthritis, painful diabetic neuropathy, neuropathic losses and changes of sensation, and other causes of leg pain such as spinal and nerve root pain are also common, particularly with age, and these disorders can obscure accurate diagnoses. Justifications of the costs of laboratory vascular investigation weigh negatively on referring medical practitioners when the likelihood of surgical vascular intervention is negligible in people without clearly vascular related symptoms.

Impairments in pressure and flow may result in tissue death, necrosis and gangrene of the affected periphery, but this outcome is not a common manifestation of PAD. In the Framingham study, only 1.6% of patients with claudication reached the amputation stage after 8.3 years of follow-up as cited by Domingez & Rowe, (2015) in an overview of PAD. A much more significant outcome therefore is the predicted mortality for patients with claudication, which is approximately 30% at 5 years of follow-up, 50% at 10 years, and 70% at 15 years. Large studies show that mortality for people with PAD is comparable to those who have already had MI or CVA (Caro et al. 2014, Mazimba 2010).

3.2.3 Current therapies for PAD

PAD is not curable but manageable, and appropriate treatment can reduce morbidity and mortality. In cases with PAD and foot ulcer, aggressive CVD management reduced 5yr mortality from 58% to 36% (Hinchliffe, et al. 2015).

3.2.4.1 Lifestyle modification
The most effective single intervention for PAD is the cessation of smoking. A recent meta analysis shows that all cause mortality in persons with diabetes is increased by 55% with smoking. Death rates from CVD are increased by 50% in smokers. Death rates of former smokers approximate those of non-smokers, so education and the use of agents to support the quitting process are well justified (Hackethal, 2015). A body of research indicates the benefits of increased activity over sedentary lifestyles on metabolic disease risk factors (Lakka, et al. 2002).

### 3.2.4.2 Pharmacological therapy

Daily aspirin is recommended for overall cardiovascular care. In addition, the following agents have shown promise in the management of PAD and may be considered: Pentoxifylline, which improves red blood cell deformability, hence its ability to perfuse the smallest vessels; Clopidogrel; a platelet aggregation inhibitor, Enoxaparin, an anticoagulant that reduces microthrombus formation; Cilostazol, which has both anti-platelet aggregation and vasodilatory actions; and statins, due to their antilipemic effects which maintain vessel lumen, reducing atherosclerotic plaque formation (Domingez & Rowe 2015).

### 3.2.4.3 Surgery

Surgical treatment options are typically reserved for patients with more severe disease or those in whom nonsurgical management fails. These options may include (1) open bypass surgery and (2) endovascular therapy (eg, stents, balloons, or atherectomy devices). Open surgery dominated the treatment options 2 decades ago, but endovascular management of PAD has become exponentially more popular since then. Along with the proliferation of endovascular procedures, there has been a decrease in amputation rates for patients with PAD. Factors directly contributing to lower amputation rates are difficult to delineate; but are likely to involve a combination of improved screening processes, better medical therapy, and evolving surgical device and technical modalities (Domingez & Rowe 2015). “A few studies have directly compared
endovascular and open surgical treatment options for patients with symptomatic PAD. A meta-analysis of 4 randomized control trials and 6 observational studies was unable to establish any well-defined superiority for either approach. Overall, recommendations for selecting a treatment modality depends on the patient’s life expectancy and comorbid conditions, as well as on the extent of the occlusive disease” (Domingez & Rowe 2015).

3.3 GTN

3.3.1 Literature search strategy

The literature search strategy for this section on glyceryl trinitrate for pedal applications is outlined. Databases used were Cinahl Plus, Ebsco Host, Medline, the Cochrane Library, Science Direct, Scopus, and Pub Med Central. Search terms used were GTN* not angina, Foot* and GTN, PAD* and GTN*, PVD* and GTN*, GTN and diabetes*, GTN * ischemia, GTN and neuropathy (the asterisk refers to Boolean search options expanded to include all other forms of the relevant words). Other versions of the term GTN, including glyceryl trinitrate, trinitroglycerin, and TNG were also used. No exclusions were made regarding the date, quality, or size of the studies. The search was restricted to English language publications. Because of the small number of studies specifically dealing with the effect of GTN on pedal perfusion, all of the papers found with that focus are presented and evaluated within this literature review.

3.3.2 Description of GTN

Other names synonymous with glyceryl trinitrate are trinitroglycerin and nitroglycerin (US National Library of Medicine, 2014). The chemically and historically related compound, nitroglycerin, is the active ingredient in the explosive substance, dynamite.

The chemical formula of GTN is 1, 2, 3-propanetriol trinitrate, an organic nitrate with a molecular weight of 227.09 with the following structural formula:

\[
\text{CH}_2\text{—ONO}_2
\]
\[
\text{CH}\text{—ONO}_2
\]
\[
\text{CH}_2\text{—ONO}_2
\]
The family of substances known as organic nitrates has biological actions that result in powerful and reliable vasodilation of blood vessels in human and animal vascular systems. This is caused by a mechanism involving nitric oxide donation. Nitric oxide was first identified in 1986 (Han, Zippin, & Friedman, 2009) and its mode of action was discovered to be a trigger of the release of endothelial relaxing factor that causes vasodilation in both arterial and venous blood vessels.

Glyceryl trinitrate remains the drug of first choice, both in the acute symptomatic relief of angina pectoris and for sub-acute and chronic management of this condition (Mayer & Beretta, 2008). In addition to the cornerstone role of GTN in the treatment of angina, there is a range of additional applications for GTN that have been documented in the literature. These applications harness its well-known properties of reliable vasodilation, and, additionally and more latterly, other less widely known actions.

3.3.3 History of the therapeutic use of Glyceryl trinitrate

Nitroglycerin was first discovered by Ascanio Sobrero in Turin, Italy in 1847. He gave a famous lecture in which he demonstrated his discovery by detonating some of the material, causing an explosion. His face was badly scarred as a result of this explosion and he went on to declare publicly his regret at being the discoverer of GTN when its use as an explosive in warfare later resulted in large-scale human suffering.

In the earliest animal experiment on this substance, GTN was shown to improve peripheral circulation by dilation of the capillaries in the frog’s foot by Richardson in 1864 (Marsh & Marsh, 2000).

Early discovery of the bioactivity of GTN on human physiology followed observations of factory workers making dynamite from nitroglycerin in the 1860s. These workers reported relief from anginal pain while at the factory site but recurrence of pain symptoms when not at work. In addition, the increased prevalence of “Monday headaches” on initial exposure to the vasodilatory effects of GTN, and a co-existing tendency for increased risk of “Sunday heart attacks” with withdrawal of the exposure to the drug, were noted (Marsh & Marsh, 2000).
A related compound, amyl nitrite, was first discovered as a treatment for angina pectoris in 1867 by Brunton. He published his findings in the Lancet (Brunton, 1867), noting the phenomenon of tolerance as pharmacological resistance to repeated doses. William Murrell described the experiments he performed on himself and 35 other subjects in detail over four successive issues of the Lancet (Marsh & Marsh, 2000). He demonstrated the effective use of GTN as an antihypertensive and antianginal agent. Many of the principles of the therapeutic use of GTN still stand unchanged since these discoveries, and the dose range he determined, ranging from 30 to 120 mg, has remained as a recommended therapeutic standard in the British pharmacopoeia to the current day (Marsh & Marsh, 2000).

Ferid Murad discovered the release of nitric oxide (NO) from nitroglycerin and its action on vascular smooth muscle in 1977. Robert Furchgott and John Zawadski recognised the importance of the endothelium in acetylcholine-induced vasorelaxation in 1980, and Louis Ignarro and Salvador Moncada identified endothelial-derived relaxing factor (EDRF) as NO in 1987 (Marsh & Marsh, 2000).

Since the discovery of its bioactive properties, GTN has been used for diverse medical disorders involving several different physiological systems. It continues to be used in a widening range of human and veterinary contexts. Two of the most recent and novel applications for GTN are its use in condoms to improve penile erection quality and duration (Futura 2011a, 20011b), and in the emergency treatment of poisonous snake bite (Van Helden, 2011)—both of which are discussed in detail below.

3.3.4 Pharmacology

Applied pharmacological knowledge regarding GTN has hitherto been focused extensively on cardiological applications (Uxa et al., 2010; Wei et al., 2011; Zimmerman et al., 2009). To date there has been relatively little application of this knowledge to the problems of perfusion to the lower limb. Glyceryl trinitrate causes a powerful and reliable physiological action of vasodilation. This vasodilatory action works on peripheral arteries and even more potently on veins (Merck Sharpe & Dohme Corp., 2014). The propensity to dilate veins needs to be studied in relation to potential application to the lower limb and effects on varicose veins and venous ulceration.
According to Mayer and Berretta (2008), the mechanism of action to cause smooth muscle relaxation involves both enzymatic activity (through mitochondrial aldehyde dehydrogenase with thiol and subsequent nitrous acid production) and non-enzymatic activity (through chemical reaction with cysteine and production of guanylate cyclase).

3.3.4.1 Routes of administration

Glyceryl trinitrate can be used intravenously for rapid reduction of cardiac load in emergency situations (Kelly, Ahmed, & Horowitz, 2005). Topical GTN can be administered for skin application in the various forms of ointment, gel, transdermal patches, transdermal tape, and spray specific for a variety of applications:

- The sublingual route is effective for rapid absorption and delivery of antianginal effects within seconds of application.
- Buccal patches are designed to adhere to the inside of the cheek or gums and can deliver higher plasma concentrations with longer action than spray GTN (Datapharm, 2011). Buccal patches have been used in cardiac treatment for coronary artery disease management in medical and in paramedical applications for cardiac emergency (Datapharm, 2011; Walker, Heer, & MacSweeney, 1999).
- Vaginal gel is used in obstetric applications for cervical ripening.
- Ointments are used in the treatment of anal fissure and Raynaud’s phenomenon (Anderson et al., 2002; Herrick, 2009).
- Adhesive dermal tape impregnated with GTN was used in measured dose strips in a study on Raynaud’s phenomenon (Kan, Akimoto, Abe, Okada, & Ishikawa, 2002).
- Transdermal patches, due to their sustained delivery, are useful for maintenance of angina management. Transdermal GTN patches have been used more recently in the treatment of tendinopathies (Hunte & Lloyd-Smith, 2005; Paoloni, 2006; Paoloni & Murrell, 2007).
- Both GTN spray and patches have been used topically via dermal application for treatment of painful peripheral neuropathy (Agrawal et al., 2007; Agrawal, Goswami, Jain, & Kochar, 2009; Rayman, Baker, & Krishnan, 2003; Toney, 2009; Yuen, Baker, & Rayman, 2002).
Patch delivery of medications has advantages, having been shown to improve compliance with medication regimens and to reduce “pill burden”. These factors are particularly important in cases of people with chronic pain (Jorge, Feres, & Teles, 2011).

3.3.4.2 Absorption

Transdermal absorption of GTN from patches ranges with five to tenfold variability between individuals (Merck Sharpe & Dohme Corp., 2014; MIMS Australia, 2015). Absorption is affected by arterial perfusion, temperature, and cardiac output (Vanakoski & Seppalla, 1998).

Absorption from transdermal patches leads to steady state blood plasma levels about 2 hours after application, being detectable at 30 minutes after application. After patch removal, plasma levels fall to half steady state levels by 30 minutes and are undetectable at 2 hours.

The effectiveness of GTN is affected by arterial perfusion which is directly related to temperature (Kan et al., 2002; Rubenstein & Sessler, 1990) being enhanced by heat and reduced when cold. Systemic absorption of transdermally and subcutaneously administered drugs including GTN, nicotine, and insulin are significantly enhanced by external heating, demonstrating higher associated GTN plasma concentrations (Vanakoski & Seppalla, 1998). This change was quantified in a study by Klemdal, Gjesdal, and Bredesen (1992) where, after 10 min of heating, the plasma nitroglycerin level increased from 3.1 to 7.6 nmol/l. Conversely, local cooling with an ice pack for 15 minutes reduced GTN plasma levels from 2.1 to 1.4 nmol/l. These researchers attributed the marked changes in absorption to changes in subcutaneous blood flow.

3.3.4.3 Metabolism

Glyceryl trinitrate is rapidly metabolised in the liver. Two active major metabolites, the 1, 2- and 1, 3-dinitroglycerols, the products of hydrolysis, appear to be less potent than are glyceryl trinitrate as vasodilators, but they have longer plasma half-lives. There is extensive first-pass deactivation by the liver following gastrointestinal absorption. The mechanism of vascular muscle relaxation is mediated by cyclic GMP (cGMP) which is the end product of

### 3.3.4.4 Actions

The potent vasodilatory action of GTN is caused by relaxation of vascular muscle in peripheral arteries. The effect of locally increased vascular pressure results in passive dilation of veins in addition to the arteries (Rossi 2015).

The effects on the venous system are more pronounced than are those on the arterial circulation. This is due to greater venous vessel lumen and decreased resistance in venous blood vessels compared with the arterial vessels. The dilating effect on the resistance vessels reduces cardiac work and myocardial oxygen consumption demand with reduction in cardiac afterload. Dilation of the vessels, including large veins, promotes peripheral pooling of blood and decreases venous return to the heart, which further reduces left ventricular end diastolic pressure. This reduction in preload further reduces the cardiac oxygen demand. Dilation of the coronary arteries also occurs (Australian Medicines Handbook 2015, Merck Sharpe & Dohme Corp., 2014).

The action of GTN causes nitric oxide donation and flow-on effects relevant to perfusion and wound healing in the foot. Nitric oxide is involved in a cascade of multiple events.

Therapeutic doses of glyceryl trinitrate reduce systolic and mean arterial blood pressures (Merck Sharpe & Dohme Corp., 2014). The pressure reduction is caused by the increased volume of the overall vascular network because of the dilated vessel lumen, with constant blood volume.

Effects of GTN may occur not just through the widely known vasodilatory action, but through other mechanisms, notably anti-inflammatory effects on blood vessels, wound healing actions, and angiogenesis of the vaso nervosorum that may address neuropathic pain and pathology.
Effects of vasodilation on peripheral vessels

Glyceryl trinitrate has been used to reverse the vasospastic constriction in the periphery in Raynaud’s disease in hands (Anderson et al., 2002; Chung et al., 2009; Herrick, 2008, 2009; Herrick et al., 2011) and in reducing leg claudication symptoms (Heer, 2001).

3.3.4.5 Elimination

Drug elimination is achieved by several different physiological pathways. They include the major processing organs of liver and kidneys, but drugs can also be cleared by the metabolising action of organs, such as the heart, skin, lungs, and by their action within blood vessels themselves. Drugs are primarily eliminated via metabolism, biliary excretion, or renal clearance of unchanged drug in the urine (Hosey, Broccatelli, & Benet, 2014). The rate of clearance of a drug can be measured by urinary content of unchanged drug or drug metabolites as well as by bile sampling in faeces to indicate hepatic clearance. GTN is eliminated at a much greater rate than can be accounted for by cardiac and hepatic clearance. Other tissues including blood vessel walls (where its primary action of vasodilation occurs) are known to be involved (US National Library of Medicine, 2014). Elimination rates are also related to hepatic function, with hemoglobin levels implicated. Elimination of GTN would therefore be expected to be reduced with increased age, liver or kidney disease, and any reduction in hemoglobin levels. Metabolites of GTN are excreted in urine and bile (US National Library of Medicine, 2014). Urine testing of metabolites is the most practical method to measure the rate of elimination of GTN (Bennett, Nakatsu, Brien, & Marks, 1984; de Berrazueta et al., 2003).

3.3.4.6 Tolerance

It is well established that nitrate tolerance is a limiting factor in the clinical response to organic nitrate administration. The mechanism of nitrate tolerance leading to the reduced responsiveness of blood vessels to organic nitrates is regarded as a complex multifactorial phenomenon (Mayer & Beretta, 2008).

The major clinical limitation of nitrate therapy, including topical GTN, is the rapid loss of haemodynamic and anti-ischaemic effects during continuous therapy (Agvald, Hammar, & Gustafsson, 2005; Uxa et al., 2010). This phenomenon exists with nitrate use in all its
administration routes. The investigation of tolerance has been limited by the difficulty of studying both nitrate plasma measurements due to the rapid breakdown of nitrates by the liver and the instability of the compound in test situations (Santoro et al., 2000).

Tolerance that develops due to thiol depletion can be reduced by drug-free periods. It has been demonstrated that the development of tolerance involves the reduced production of cGMP as ALDH2 is converted to an inactive form in the bioactivation of GTN. Nitrate tolerance is also likely to be the result of endothelial dysfunction triggered by GTN-derived superoxides. Such a mechanism sets a ceiling on the efficacy of GTN as a function of dose and duration of treatment (Wei et al., 2011).

Standard treatment regimens for standard doses of transdermal GTN patch therapy involve intermittent therapy to allow a nitrate-free period of 10–12 out of every 24 hours to preclude development of tolerance (Australian Medicines Handbook, 2015, MIMS Australia, 2015). Periods of 10-12 hours are effective, and shorter periods have not been well studied (Merck Sharpe & Dohme Corp., 2014).

Recent additional work suggests that tolerance is significantly dose-dependent and that small doses below 2.5mg avoid the development of tolerance to the drug and may have haemodynamically identical effects to larger doses (Uxa et al., 2010; Wei et al., 2011).

### 3.3.4.7 Doses

There are no studies addressing doses of GTN for PAD in the literature to date. Therefore the only available studies discussing GTN doses in the periphery were used as guidance. These included Rayman et al. (2003) in their study about the effect of GTN on peripheral neuropathy, and six studies about the effect of GTN on tendinopathy with Paolini (2004-9) as the first author.

The work of Paolini and his colleagues (Paolini, Appleyard, Nelson, & Murrell, 2005; Paolini & Murrell, 2007; Paolini, Murrell, Burch, & Ang, 2009) contributed to the determination of doses used in the study. With regard to lower GTN thresholds, they found that in cases of chronic noninsertional Achilles tendinopathy, a dose of 1.25 mg was effective (Paolini et al., 2005). The continuous use of low doses of 1.25 mg and 0.74 mg were also effective for epicondylitis (tennis elbow), but two higher doses (1.44 and 3.6 mg) were not (Paolini & Murrell, 2007). In their 3-year follow up of GTN on Achilles insertional tendinopathy, a wide range of doses and application
regimens was used, from 1.25 mg/24 hours to 5 mg/24 hours. Applications varied from 8 hours a day to continuous patch application. The researchers concluded that all GTN users experienced reductions in pain symptoms despite varying doses and regimens (Paoloni et al., 2009). A summary of the literature regarding continuous treatment indicates that higher doses do not provide better results. Higher doses quickly exceed the tolerance threshold for continuous treatment, then lose all effect, as demonstrated by both Wei et al. (2011) and Paoloni and Murrell (2007).

In establishing the maximum dose for this study, the issue of tolerance predominated. The problem of tolerance with GTN means that doses above 2.5 mg require a break in their use after about 14 hours, with a rest period of about 10 hours to reinstate drug effect. This is because of depletion of thiols in the biochemical pathways of action. To find a therapeutic dose for wound healing in ischaemic feet, the dose that would provide uninterrupted and extended improvement in perfusion for an individual would be ideal, with effects maintained consistently across a full 24 hours. A dose below the reported threshold of tolerance was considered most suitable as it could be worn constantly for continuous provision of its vasodilatory and other effects. The 2.5 mg dose was reported to be the maximum dose that did not result in tolerance according to Wei et al. (2012). However, this was determined in the context of doses for angina, and therefore was tested on the chest. This dose was also used on feet of people with diabetes as recommended by Rayman et al. (2003) in their regimen for the treatment of PDN. These authors recommended titration down to 2.5 mg for each foot after a test of 5 mg on one foot for side effects. The dose of 2.5 mg was chosen as the larger dose for this study due to this evidence.

GTN is known to be generally well tolerated (Jorge, Feres, & Teles, 2011; Merck Sharpe & Dohme Corp., 2014). When side effects do occur, the commonest are headache, flushing, and dizziness. Side effects are dose related, but the low dosages used in this study, applied to an extremity, were expected to produce little or no systemic effect and therefore there would be a low occurrence of side effects (see Herrick et al., 2009; Santoro et al., 2000; Uxa et al., 2010).

In a recent meta-analysis of RCTs about the use of nitrates in stable angina, comparing doses and intermittent versus continuous treatment, Wei et al. (2011) concluded that continuous administration of low dose nitrates was more effective than were high doses used intermittently. A sustained treatment effect was provided by continuous use of low doses. Increased dose did not produce an increased benefit. The use of a small dose 1/12 the size of a standard dose was found to provide these benefits. This is of potential clinical relevance for wound healing applications if doses can be kept below the tolerance threshold.
and thereby provide 24 hour effects with continuous use. Wei et al. indicate that this threshold dose is 2.5 mg.

In support of interest for low dose applications of GTN, Uxa et al. (2010) compared a standard dose of 120 mg of transdermal glyceryl trinitrate with a low dose of 10 mg and found identical haemodynamic effects with both doses. However, the low dose was superior in that it caused neither tolerance effects nor endothelial dysfunction.

Researchers have demonstrated that the correct dose is critical for the effectiveness of transdermal GTN patches in tendinopathy. (Paoloni, Appleyard, Nelson, & Murrell, 2004, Paoloni, 2006) and others (Hunte & Lloyd-Smith, 2005), treating tendinopathy with transdermal GTN, have been using 1.25 mg patches in their studies producing statistically significant positive outcomes. However, there is agreement that further work is needed to determine the most effective dosing regimens (Hunte & Lloyd-Smith, 2005; Paoloni, 2006; Paoloni et al., 2004). The results of GTN therapy for elbow epicondylitis contributed to the trialling of new doses, showing a positive but just significant effect ($p = 0.04$) when the smallest previously effective dose was halved (Paolini, Murrell, Burch, & Ang, 2009). In a subsequent study, Paoloni (2009) extended the dose range to include the very small dose of 0.74 mg and the larger doses of 1.44 mg or 3.6 mg. The two larger doses were ineffective. Previous investigators have shown effects of GTN on epicondylitis at a dose of 1.25 mg (Andres & Murrell, 2008). The combination of these results points toward an effective dose range of 0.74–1.25 mg for this particular condition.

There is Level II evidence (NMHRC classification from 3 randomised controlled trials) that GTN therapy is effective for improving chronic tendinopathy when added to a regimen of best practice rehabilitation (Paoloni, 2006). This suggests that there is a combined treatment effect of both analgesia in the short term, and promotion of healing in the longer term when GTN is used in tendinopathy. The extent of this work helps to shed light on the question of ideal doses for the use of GTN in podiatric problems.

One could infer that dose titration is ideal on a case-by-case basis due to the wide variations of 5–10 fold in individual absorption (Australian Medicines Handbook 2015, Merck Sharpe & Dohme Corp., 2014; MIMS Australia, 2015). This would be particularly
important in cases of impaired vascular supply in the periphery because absorption is related to or dependent on vascular perfusion and cardiac output. Titrating the dose for individual effectiveness is recommended and is described by Rayman, Baker, and Krishnan (2003).

Rayman et al. (2003) suggested the following titration procedure to determine the effective dose regimen for use in painful diabetic neuropathy (PDN): initially, a trial of a 5 mg dose on one foot only; if no side effects are induced, increase the dose to 5 mg bilaterally on each foot; if side effects are triggered from the initial dose, titrate the dose down to 2.5 mg on one foot only. They describe an effectiveness of 44% treating 18 cases of PDN with this regimen.

In research about topical preparations for pain relief, Jorge, Feres, and Teles (2011) compared 2% nitroglycerin ointment with patch therapy. Ointment application extruded from a tube to a length of 2.5–5 cm is recommended, to be applied each 4–8 hours. The effect commences in 30 minutes and lasts up to 6 hours. According to Jorge et al. (2011, p. 19), “measurement of dose is hampered, especially in ointments, which become less safe when compared to the transdermal patch mode of drug delivery”.

### 3.3.4.8 Local versus systemic effect

The question arises as to whether a local effect, systemic effect, or both are produced by topical GTN. A summary of the available literature suggests that a predominantly local effect is definitely produced by transdermal GTN, and that systemic effects can also be produced. The prescribing information in MIMS Australia (2012) states that for the management of angina, patches should not be used on the periphery. This is presumably due to an attenuated central effect if the patch is placed on a peripheral anatomical location.

Experiments by Anderson et al. (2002) and Gjesdal et al. (1985) suggest that GTN used on extremities does not induce a systemic effect as it does when placed on the upper trunk for angina treatment. This issue was addressed in the study by Anderson et al. with 10 Raynaud’s cases, measuring three different adjacent fingers with laser Doppler imaging in each subject: one with GTN ointment treatment, one with placebo ointment, and one not
treated. Low topical doses of 2% ointment produced a significant \( p < .001 \) local effect on the treated finger without any effects on neighboring fingers or any systemic effects.

Francis, Hubbard, and Johnston (1977) reported a wide range of changed perfusion pressures after application of one inch of 2% topical GTN ointment to the medial aspect of the right foot and the great toe in 20 subjects. High individual variations from 0% to 400% on the treated side and 0% to 300% on the untreated side were recorded. Their results demonstrated greater local effect on the treated side and some strong bilateral effects in individual cases where the effect was greatest.

Glyceryl trinitrate is otherwise known as nitroglycerin in its applications as an explosive, is the active ingredient in gunpowder. In a study of gunpowder factory workers occupationally exposed to GTN, this study produced three points relevant to biomedical applications of GTN:

- Tenfold individual differences in GTN absorption were demonstrated within a small sample of 12 subjects.

- The anatomical areas in occupational contact with the compound (arms despite gloves and clothing) had tenfold to 100 fold higher local concentrations in blood plasma than an unexposed body part (the groin).

- GTN exposure was associated with headaches, demonstrating a systemic effect of vasodilation in addition to the local concentrations demonstrated by the location specific plasma sampling. (Gjesdal et al. 1985).

The recently validated peripheral applications of GTN for snake bite in delaying perfusion of poison from the periphery to the central organs indicates that the effect of local vasodilation functionally outweighs systemic vasodilatory effects. See Section 3.3.5.8 below for detail.

In summary, the literature indicates that both local and systemic vasodilatory effects can be derived from the application of topical GTN. The distinction of these various effects depends on the absorbed dose which is influenced by skin temperature, which is in turn
influenced by variations in vascular perfusion and the ambient environmental temperature. Individual differences which are as yet undetermined, and the anatomical location of the GTN exposure are also contributing factors in the manifestation of local versus systemic effects.

3.3.5 Applications

The uses of GTN in medicine span several physiological systems. Due to its long history and predictable, powerful vasodilatory action, while being known as a drug that is generally well tolerated and rapidly eliminated, GTN is regarded as a safe, inexpensive, and reliable pharmacological agent (Heer, 2001). Although its use in cardiac medicine is highly developed, the full potential of GTN as a therapeutic agent in its many other applications is yet to be explored. The research into GTN use in tendinopathy by Paoloni and colleagues (Paoloni, Appleyard, Nelson, & Murrell, 2005; Paoloni & Murrell, 2007; Paoloni, Murrell, Burch, & Ang, 2009) is notable for its detail in determination of specific small, effective doses. See Section 5.1.2.

3.3.5.1 Cardiology

Glyceryl trinitrate is the first line treatment for angina pectoris and is the most useful of the organic nitrates or nitrovasodilators, a class of compounds that have been used since the initial discoveries in 1867 for the treatment of coronary artery disease (angina pectoris), congestive heart failure, and myocardial infarction. Medline lists more than 15,000 publications about GTN and other organic nitrates relating to cardiological applications (Mayer & Beretta, 2008).

3.3.5.2 Vascular therapy, surgery, and research

The predictable vasodilatory effect of GTN is frequently used in vascular research to compare GTN mediated vasodilation with other pharmacological and surgical interventions. Glyceryl trinitrate has proved to be effective in causing vasodilation in both diseased and normal blood vessels (Azizi et al., 2010) associated with increased vascular supply distally in post stenotic zones of prior ischemia after vascular surgery (Owens et al., 2009; Ray, Buckenham, Belli, Taylor, & Dormandy, 1997). It has also been used to improve vessel patency after arterioplasty (Ray et al., 1997) and it is helpful in preventing phlebitis
associated with the failure of intravenous catheterisation sites (dos Reis, Silveira, Vasques, & de Carvalho, 2009; Tjon & Ansani, 2000).

Improvement in intermittent claudication, using sublingual GTN has been associated with positive results ranging from a 9% to 19% increase in maximal treadmill walking distance (Heer, 2001).

3.3.5.3 Protective effects on the cardiovascular system

A cardio protective effect has been described from nitrates, and this persists beyond the development of vascular tolerance: “Nitrates exert a direct myocardial anti-ischemic effect that is independent from their vascular actions” (Csont & Ferdinandy, 2005, p 57).

Findings of de Berrazueta et al. (2003) about the effects of GTN in PAD indicated highly significant ($p < .0001$) biochemical CVD risk marker improvements (C reactive protein) in addition to vasodilatory benefits. The suggested mechanism involves generation of nitric oxide (NO), which they suggest effectively stabilises plaque in the atherosclerotic arterial wall by an anti-inflammatory effect on the vessels. Their study demonstrated a vascular anti-inflammatory effect of 15 mg transdermal GTN patches applied to the anterior face of the thigh. “This may provide a new therapeutic approach to understanding the efficacy of nitrovasodilators in the improvement of atherosclerotic syndromes” (de Berrazueta et al., 2003, p. 5). These researchers indicate that more studies are needed to evaluate the apparent positive clinical and biochemical effects of GTN on PAD.

3.3.5.4 Pain

Analgesic properties reported in both ischemic pain and neuropathic pain are of therapeutic interest and great potential value. In both these conditions, pain control is notoriously difficult and the agents available are often poorly tolerated, often only partly effective, and also expensive (Jorge et al., 2011; Scimeca, Fisher, Bharara, & Armstrong, 2010). Apart from a few articles, the use of GTN for pain is largely unexplored and untapped. Chronic pain is strongly associated with reduced quality of life (Brophy, 2006; O’Connor, 2009; Wu, Wrobel, & Armstrong, 2007). Pharmacological treatments in the areas of ischemic and
neuropathic pain are associated with limited effectiveness, high side effect profiles, high costs, and poor long-term compliance (Brophy, 2006; Wu et al., 2007).

In their review about pain relief, Jorge et al. (2011) include transdermal GTN with the comment that “since topical nitrates are devoid of renal, gastrointestinal, and hematological adverse effects, they can be considered for the treatment of local pain, especially in patients contraindicated for NSAID use” (p. 19).

Analgesia has been also noted in the applications of GTN in tendinopathy. Paoloni (2006) and Hamza et al. (2010) noted that nitric oxide is negatively correlated with pain during acute inflammation in a study on the pain of oral dental surgery.

3.3.5.4.1 Ischaemic pain

Pain from ischaemia was reported to be significantly relieved by topical GTN in a paper by Fletcher, Wright, Wilkinson, Farr, & Sellars (1997), who studied 15 people with end-stage PAD in chronic pain that was not managed by opiates. Fourteen of these people experienced good to moderate relief from pain with the addition of GTN to their therapy and were able to reduce oral medications, discontinuing opioids to be managed by codeine and paracetamol combinations. Further research in this area was recommended by these authors. However, there appears to be an absence of subsequent literature on this topic.

3.3.5.4.2 Neuropathic pain

Available pharmacological therapy for peripheral neuropathy is underused or discontinued in two thirds of patients due to ineffectiveness, adverse events, or the ongoing costs of therapy (O’Connor, 2009). In a review of the prevalence and severity of PDN, Brophy, Davies, Taylor, and Williams (2006) stated that 26.4% of people with diabetes have PDN and only 50% have ever tried or been offered treatment. Eighty percent of these people have severe or moderate pain that significantly impacts quality of life. They estimate therefore that 500,000 people in the UK suffer from PDN. This high prevalence indicates the importance of effective treatment for this condition.
A recent systematic review by Dworkin et al. (2010) does not include GTN as an agent for neuropathic pain relief. However, some literature does exist suggesting its effectiveness. The systematic review by Veves, Backonja, and Malik (2008) about PDN refers to topical nitrates and includes the studies by Yuen, Baker, and Rayman (2002) and Rayman et al. (2003) that demonstrate positive results from the use of GTN spray for PDN. The Yuen et al. study of 22 people was notable in that 50% of chronic nonresponders to other analgesic treatment for PDN experienced a reduction in pain with GTN spray. This figure was similar to the 44% reported by Rayman et al., in their study of 18 people using GTN for PDN.

Glyceryl trinitrate spray was shown to be effective as a treatment for PDN in two studies, first of 48 then another of 83 subjects by Agrawal et al. (2007) and Agrawal, Gosami, Jain, & Koshar (2009). Both of these trials produced positive results with the number needed to treat (NNT) being 4 and 5 respectively. Sodium valproate and GTN were used alone and in combination in the second study with the best results being NNT 4 for sodium valproate alone in the second study. The conclusions were that both drugs are well tolerated and provide significant improvement in pain scores as well as in electrophysiological parameters of nerve conduction tests and sensation.

The mechanism of action responsible for GTN’s therapeutic effects on neuropathic pain is not yet known, but an anti-inflammatory effect is proposed. “It has a direct analgesic effect in the peripheral nervous system, mimicking the actions of acetylcholine at the nociceptor level” (de Berrazueta et al., 2003, p. 6). Angiogenesis of the vasa nervosorum is a mechanism proposed by Agrawal et al. (2007).

### 3.3.5.5 Anal fissure

Glyceryl trinitrate is a first-line drug for the healing of anal fissure, and can be purchased over the counter for this purpose. Rectogesic is a preparation of 0.2% GTN ointment and is also available in the form of wipes, without prescription, for relief of hemorrhoids and anal fissure. Glyceryl trinitrate is included in a systematic review of treatment for anal fissure (Nelson, 2004). Healing of anal fissure is complicated by extended sphincter contraction and the associated reduced vascular supply (Sokol, 2014).
Equal efficacy was found for the healing of anal fissure between three groups undergoing treatment with GTN cream, patches, and surgery (Zuberi, Rajput, Abro, & Shaikh, 2000). However, a systematic review indicated that surgery was much more effective than was GTN in healing anal fissure (Nelson, 2004).

3.3.5.6 Obstetrics

Glyceryl trinitrate and other nitro vasodilators have been used for acute uterine relaxation for over 120 years. A total of 32 studies concerning this topic are reported in the English language literature (Caponas, 2001). There are indications for the use of GTN in achieving rapid uterine relaxation in the antepartum (for pregnancy termination procedures), intrapartum, and postpartum periods. The consensus is that safety of GTN in obstetric emergencies is high, and no adverse maternal or neonatal outcomes have been reported (Nunes et al., 2006; Redick & Livingston, 1995).

3.3.5.7 Erectile function

Futura Medical, the makers of Durex condoms, has developed a condom containing GTN in its tip. It is effective in improving penile erection by enhancing the vasodilatation in the glans of the penis in response to contact with local topical GTN. Up to 22% of healthy men reportedly experience loss of erection while wearing a condom due to loss of sensitivity. This contributes to reluctance to use condoms despite their safe-sex benefits. Improvements have been demonstrated with both male and female sexual partners, noting the enhanced experience over standard condoms. A significant proportion of both men and women also felt that the GTN condom increased penis size, and a significant proportion of women reported a longer lasting sexual experience. The developers are promoting its use as an aid to contraception and the practice of safe sex (Futura, 2011a). Eighty eight percent of current condom users and 49% of noncondom users were interested in purchasing these condoms, according to market research.

Futura Medical has also recently developed a topical gel for erectile dysfunction using GTN (Futura, 2011b). With the most conservative predictions, the number of men with erectile dysfunction is expected to more than double from 152 million in 1995 to 322 million by 2025. Fifty two percent of men aged 40 or over have some degree of erectile
dysfunction (Araujo, Johannes, Feldman, Derby, & McKinlay, 2000). Market research indicates that only one in five men with erectile dysfunction seek medical advice. All existing treatments for erectile dysfunction require a doctor’s prescription. The proposal to market GTN for erectile dysfunction treatment, available without a doctor’s prescription, may bring it into widespread use.

3.3.5.8 Snake bite

The mode of action in snake bite therapy relies on local vasodilatory action. When applied topically in an ointment to the periphery around the bite it slows the absorption and circulation of the toxin, thereby ameliorating and delaying its effects (Van Helden, 2011). Recent research suggests that application of GTN ointment can delay by 300%, the speed with which venom travels from an extremity via the lymphatic system, prolonging survival times from otherwise fatal envenomation by 150% in animal and human trials, thereby allowing more time to reach emergency services. In both animal and human experiments, GTN ointment significantly increased the time taken for venom to reach central organs. It is proposed for inclusion in first aid kits for use in conjunction with standard treatments for snake bite (Van Helden, 2011).
3.3.5.9 Equine applications

Glyceryl trinitrate has been used for many years in the treatment of laminitis, a condition of the horse’s hoof that shares some physiological elements of lower leg compartment syndrome seen in humans. Topical preparations of GTN have been used with the mechanism of reversal of the ischaemia that can lead to tissue destruction in the closed environment of the hoof (Hinkley, Fearn, Howard, & Henderson, 1996; Simon, Bailey, & Elliot, 2004). Hinkley et al. (1996) concluded that GTN is effective in increasing the blood supply within the hoof of laminitic ponies. Hoff, Hood, and Wagner (2002) concluded the opposite. However their methodology involved cooling the hooves by standing the horses in iced water first. They did note that GTN absorption had occurred by general hypotension. These contrasting results confirm the findings of other studies that the absorption of GTN is highly temperature dependent (Barkve, Langseth-Manrique, Bredesen, & Gjesdal, 1986, Coakley, 1983; Klemsdal, Gjesdal, & Bredesen, 1992), as presented in section 3.3.6.5.

3.3.5.10 Pedal and peripheral applications

The following section summarises the literature regarding the use of GTN describing the effects specifically on the extremities, and particularly on the toes and feet.

3.3.5.10.1 Ischaemia, healing, and amputation

The major etiological drivers of lower limb amputations are the vascular and neurological complications of diabetes. Vascular complications of diabetes continue to account for much of the end-stage pathology of chronic ulcerated wounds that contribute to the scenarios precipitating amputation (Rogers et al., 2010).

The link between reduced toe brachial indexes (TBIs) and diabetic ulceration and amputation has been demonstrated in a study of 65 limbs in 40 cases with great toe pressures less than 40 mm Hg (Vatharajan, Pillay, Hitos, & Fletcher, 2006). Table 3.1 shows links with low toe pressures (TPs) and amputation prognosis. Similarly, Carter et al. (1993) have linked amputation prognosis and healing outcomes with toe pressures with and without diabetes. See Table 3.2.
Table 3.1

Amputation Prognosis of Low Ankle and Toe Pressures

<table>
<thead>
<tr>
<th>Vascular index</th>
<th>Major amputation</th>
<th>Minor amputation</th>
<th>No amputation</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP mmHg</td>
<td>13.0</td>
<td>20.3</td>
<td>23.6</td>
</tr>
<tr>
<td>TBI</td>
<td>0.08</td>
<td>0.14</td>
<td>0.18</td>
</tr>
<tr>
<td>ABI</td>
<td>0.16</td>
<td>0.48</td>
<td>0.54</td>
</tr>
</tbody>
</table>

* Source: Vatharajan et al. (2006)

Table 3.2

Relationship of Toe Systolic Pressure to Healing Prognosis

<table>
<thead>
<tr>
<th>Toe Pressure mm Hg</th>
<th>Probability of healing (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No diabetes</td>
</tr>
<tr>
<td>Below 20</td>
<td>25</td>
</tr>
<tr>
<td>20–30</td>
<td>73</td>
</tr>
<tr>
<td>30–55</td>
<td>100</td>
</tr>
<tr>
<td>Above 55</td>
<td>100</td>
</tr>
</tbody>
</table>

* Source: Carter et al. (1993).

3.3.5.10.2 Vasodilation

Francis, Hubbard, and Johnson (1977) performed a study involving 20 randomly selected hospital inpatients with whom only the right hallux was treated with 2% GTN in Nitro-Bid ointment. Changes in pulse volume were measured with plethysmography. Measurements were taken of both feet, and differing effects were noted on both the left untreated limb and the right treated limb. Changes ranged from 0%–400% increases in pulse volume on the treated side. Sixteen of the 20 subjects had some increases in pulse volume on the left foot also, demonstrating a highly variable but primarily local effect. These changes ranged from 0%–300% increases on the nontreated side. In summary, GTN was found to be effective as
a vasodilator in the lower extremities, with some effect on both feet in most people (80%). In 85% of subjects (17 out of 20), GTN more than doubled the pulse volume of the right (treated) foot. In addition, 30% of subjects had a doubling on the left foot.

Although Francis et al. (1977) published in a well-known podiatry journal and made recommendations for the further research and use of GTN, no further podiatric-specific literature about the use of GTN was available until publication of a paper by Kim et al. (2006). These researchers performed a trial on healthy podiatry students to test for increased perfusion by measuring temperature changes and changes in plethysmographic (PG) toe pressures. The overall temperature of all feet in the study dropped and no significant changes were reported in PG measures. However, their methodology contained several flaws that invalidated their results. Seated subjects were positioned with their feet elevated despite lying flat being the ideal position recommended for evaluation of pedal and lower limb perfusion to prevent errors due to hydrostatic pressure (Gornick et al., 2008). Elevating the lower limb can cause up to a 50% reduction in vascular perfusion pressure according to a Swedish study (Wiger & Styf, 1998). Further flaws were present in their methodology:

- Socks and shoes were removed, then immediate measurements of temperature were performed without temperature acclimatisation.
- The subjects were not at rest but moved between three stations where patches were applied and removed, temperatures taken, and PG readings performed.
- No rest times before measurement were reported or appear to have occurred.
- The patches were removed some time before the final temperature and PG measures were taken.

Kim, Ballinger, and Kushner (2006) acknowledge that ambient temperatures under which this experiment was performed could have influenced results significantly, but did not report them. They concluded that GTN failed to cause changes in temperature, despite the fact that the temperatures in the GTN treated feet were warmer than the control treated feet at the end of their study.
Sawka and Carter (1992) report significant temperature effects of toe pressure measurements taken between 10 °C compared with 30 °C, noting that pressures increase with increasing temperatures and that cold temperatures reduce pressures and create an unreliable test environment. Hoyer et al. 2013b, in his review of toe pressures for the diagnosis of PAD, stratified studies into those that used heating and those that did not, concluding that studies using heating provided the most reliable and accurate results.

Loss of sensitivity to cold is related to age and arterial insufficiency. Protection from the effects of cold, despite the lack of awareness that characterises this loss of sensation, is important in the holistic management and clinical treatment of aged people with reduced vascular supply. Further research on the effects of GTN therapy should aim to control for seasonal climate and environmental temperature changes (Chung et al., 2009, Cloete et al. 2009, Hoyer et al. 2013, McAra, 2011c).

Rectogesic was mentioned earlier as an over-the-counter preparation of GTN specifically designed for anal fissure. It can also be used for the prevention and treatment of chilblains (erythema pernio), to be applied sparingly to the skin of affected extremities, while wearing gloves to limit absorption to the desired location and dose. It is recommended to be used once daily initially then titrated to response. Rectogesic is anecdotally used in this way to manage cold extremities by people such as ski field workers, outdoor tradespeople, and farmers when they are working in cold environments.

The clinical presentation of chilblains and associated arterial ulceration should be considered in conjunction with loss of sensitivity to cold and its perception related to age and neurological and arterial insufficiency. Protection from the effects of cold, despite the lack of awareness that characterises these losses of sensation, is important in the holistic management and clinical treatment of aged people with reduced vascular supply. Further research on the effects of GTN therapy should aim to control for seasonal climate and environmental temperature changes (Chung et al., 2009; McAra, 2011c).
3.3.5.10.4 Raynaud’s phenomenon and systemic sclerosis

Raynaud’s phenomenon is a vasospastic disorder causing blanching ischemia to the extremities. It can be idiopathic or associated with other disorders such as connective tissue disease and scleroderma. Glyceryl trinitrate has been shown to be effective in Raynaud’s phenomenon and in systemic sclerosis (Aikimbaev et al., 1996; Anderson et al., 2002; Herrick, 2009). Herrick et al. (2011) used modified release sildenafil (Viagra) for the same purpose.

Local vasodilatory effects have been demonstrated in Raynaud’s disorder and systemic sclerosis by Teh et al. (1995) and Anderson et al. (2002). Anderson et al. showed that topically applied GTN ointment was an effective digital vasodilator in both Raynaud’s sufferers and in healthy subjects. The doses used did not cause any systemic effects as evidenced by the comparison of adjacent fingers which were untreated and did not vasodilate during the study.

Teh et al. (1995) found GTN to be effective in treating both primary and secondary Raynaud’s phenomenon, but concluded that the incidence of headaches would limit the use of GTN for this condition.

In an RCT, Chung et al. (2009) used a preparation of a 0.9% GTN gel that demonstrated a 14% improvement in Raynaud’s condition when used four times daily on fingers of Raynaud’s sufferers. The effects of ambient temperature on their results were highly significant ($p = 0.007$).

3.3.5.11 Healing

Nitroglycerin ointment is listed as a therapeutic agent for cutaneous vasodilation for the treatment of Raynaud’s disease and in the healing of trophic ulcers (Gilman, Goodman, & Gilman, 1985, p. 809).

In their article on emerging drugs for diabetic foot ulcers, Petrova and Edmonds (2006) cite angiogenic stimulants and nitric oxide donors among the agents that should be considered when correction of extrinsic factors have failed to heal a diabetic foot ulcer.
In addition to the better known vasodilatory effects associated with GTN application, several other influences are documented in the literature. The following largely sequential actions of GTN are involved in wound healing:

1. Vasodilation from endothelial relaxing reaction
2. Increase in blood vessel permeability
3. Promotion of angiogenesis
4. Stimulation of release of epidermal growth factors
5. Anti-inflammatory effects (de Berrazueta et al., 1994; de Berrazueta et al., 2003)
6. Trigger of release of chemotactic cytokines causing antimicrobial effects

An in-depth exploration of these potentially useful effects is beyond the scope of this review. However, examples of the research including some human case studies and the evidence for effective use of GTN from animal experiments are presented.

3.3.5.11.1 Case studies

In 1948, Lund published a paper stating that positive results had been found clinically in cases where peripheral vasodilation was advantageous. He cited the use of 2% nitroglycerin ointment in “30 cases of pregangrene and decubital sores in arteriosclerotic patients” and its effective use in 17 Raynaud’s patients. His report included effective treatment of acrocyanosis, intermittent claudication, chillblains, and frostbite. These reports are uncontrolled individual case observations (Lund, 1948).

Wheeland, Gilchrist, and Young (1983) described the effectiveness of nitroglycerin ointment for the treatment of severe ischemic digital ulceration in three cases. Case 1 involved a 60-year-old male tobacco smoker with ulcers on all 10 fingers, two of which had bone protruding from the wounds. Case 2 was a 68-year-old female undergoing chemotherapy for hepatic carcinoma with a 3-month history of a nonhealing ulcer with protruding bone on one finger. The third person had a history of two myocardial infarctions and triple bypass surgery. Two fingers on one hand were superficially ulcerated and the hands were cool to touch.
The first case was reportedly 50% improved within 2 weeks and healed within 4 weeks of 2% nitroglycerin ointment therapy every 4 hours or as often as tolerated. The second case was similarly 50% improved at 2 weeks and completely healed on review at 6 weeks. The final case was similarly completely healed at 1 month. These authors concluded with the recommendation that GTN be considered when treating digital ulcers (Wheeland, Gilchrist, & Young, 1983).

3.3.5.11.2 Nitric oxide

Nitric oxide is essential to life but it can be toxic and fatal in high concentrations. When early experiments first isolated nitric oxide as a colourless gas, several chemists died as a result of intended or accidental inhalation. High concentrations of NO are associated with fatal outcomes in conditions where the concentration of NO far exceeds naturally circulating levels, triggering conversion of NO to toxic compounds- superoxides, and peroxynitrite (Beckman & Koppenol, 1996).

Infection triggers NO production by sustained enzyme release, where its effects last for a few hours and are involved in physiological mechanisms that are toxic to invading pathogens. Longer term high NO levels are seen in severe disease states where the body is being overwhelmed by infection such as in septic shock and meningitis.

Nitric oxide is released in high concentrations in any condition resulting in ischemia to an organ. These include cerebrovascular accident and myocardial infarction.

Being a ubiquitous cell messenger in all vertebrates, where it modulates blood flow, thrombosis, and neural activity, NO at concentrations within physiological ranges is rapidly absorbed by various physiological “sinks”, such as the oxyhaemoglobin in red blood cells, and in vascular smooth muscle, where it is diffused intracellularly in seconds by these in vivo reactions (Beckman & Koppenol, 1996). The half life of NO in tissues is 3–6 seconds, and in blood, 1–2 seconds.

The effects of NO are known to be strongly mediated by localisation, and can be therapeutic when localised, but may be potentially toxic or undesirable when systemic. Examples of this include peripheral vasodilation that is therapeutic to reverse systemic hypertension but could cause vascular hypotension and collapse in large enough doses.
Additionally, reduction to sun sensitivity is seen, with increases of melanocyte activity in animal studies with supplementary doses of NO. Whether NO reduces DNA damage in sun exposure, or deleteriously blocks apoptosis of mutated cells remains under investigation (Weller 2003).

Weller (2003) stated that using the skin as a target organ for delivery manages many of the potential concerns of systemic overexposure to NO, and that topical administration provides opportunities to deliver drugs “directly to the site of action, at an effective dose, and usually with clinically insignificant systemic absorption” (page 533). Weller (2003) considers the potential of NO donor use in diabetic ulceration to be an exciting possibility.

It is not a conceivable risk that transdermal patch doses of GTN can donate sufficient NO to contribute to pathological NO levels. Short-term deleterious side effects most commonly include headache and less commonly hypotension, both of which are rapidly reversed within minutes by patch removal.

Nitric oxide donation by topical GTN may positively influence many of the processes of healing listed in the cascade of healing events in the previous section where any lack of available nitric oxide (NO) is a limiting factor. Supplementary GTN is proposed as facilitator in wound healing due to its actions as a NO donor (Bohl Masters et al., 2002). Nitric oxide is known to be reduced in diabetic wounds (Schaffer et al., 1997; Schaffer, Bongartz, Fischer, Proksch, & Viebahn, 2007). Nitric oxide supplementation has been proven to have significant effects on wound healing in animal studies in both nondiabetic and diabetic animals (Schaffer et al., 2007). Glyceryl trinitrate’s mechanisms of action involves its role in cell apoptosis and as a promoter of angiogenesis. Angiogenesis of the vasa nervosorum is cited as the likely mechanism for GTN in reducing pain and increasing nerve function in neuropathy (Agrawal et al., 2007), as noted above in the section on neuropathy.
Although NO supplementation to address NO deficits has been helpful in wound healing, conversely, high levels of NO have been found to impair healing (Rizk, Witte, & Barbul, 2004).

The findings of a review of NO in wound healing attributed the beneficial effects of NO on wound repair to functional influences on angiogenesis, inflammation, and other cellular processes (Luo & Chen, 2005). Luo et al. (2009) tested the hypothesis that topical NO donors may improve diabetic wound healing. This involved both in vivo and in vitro research on diabetic rats and human umbilical cells, and revealed positive effects of NO donors in facilitating diabetic wound healing.

An antibacterial effect has been found to occur with NO donors (Ghaffari, Miller, McMullin, & Ghanary, 2006; Xiong, Elson, Legarda, & Leibovitch, 1998). It is postulated by Ghaffari et al. that NO interference with bacterial mitochondrial chain respiration produces this antibacterial effect.

Hyperbaric oxygen therapy is effective due to the donation of NO from the hyperbaric oxygenation of subjects in both local and systemic pathways. Local oxygenation effects from the environment surrounding the wound, combined with oxygen-rich breathing air, provides the NO boost that enhances wound healing (Boykin, 2000; Boykin & Baylis, 2007; Efraiti et al., 2009; Latham & Kulkarni, 2013). In support of previous work investigating the mechanisms of wound healing associated with NO, Latham and Kulkarni (2013) cite angiogenesis and antibacterial effects of hyperbaric oxygen therapy. This therapy is a health fund rebatable treatment due to the strong evidence base for its effectiveness in wound healing.

As they share the common mechanism of nitric oxide donation, a cost comparison with hyperbaric oxygen therapy and GTN patch therapy may be juxtaposed to explore potential economical advantages in reducing the huge financial burden of chronic wound care. Glyceryl trinitrate costs around $30 AUD per month for a 2.5 mg dose for both feet. In comparison, hyperbaric treatment typically costs several hundreds of dollars per session (Harris, 2011). A course of hyperbaric wound treatment requires multiple sessions. Hyperbaric oxygen therapy is reserved for severe wounds due to its cost. Another
disadvantage of hyperbaric treatment is that it is available in major centers only. The potential of GTN therapy to fill a role for low cost, effective, accessible, adjunctive wound care could have profound implications for people with chronic foot wounds and for the delivery of worldwide health care in this field. This could be particularly relevant for rural and remote communities for whom hyperbaric treatment is difficult to access.

3.3.5.11.3 Experimental wound healing

Acute wounds caused in animal wound healing experiments are different in many ways from the chronic wounds typical in diabetic foot ulceration and ischemic lesions due to PAD. However, in all wounds, the cascade of events involving vessel thrombosis, homeostasis, inflammation, collagen deposition, angiogenesis, and remodelling are present.

A positive effect was demonstrated by improved healing rates in experimentally wounded mice treated with NO in high and low doses. The granulation and scar tissue in treated mice compared with controls was significantly thicker at 8 and 15 days (Schaffer et al., 2007).

In an experiment designed to mimic the clinical scenario of people with diabetes who are normoglycemic due to their diabetes control regimen, experimentally wounded normoglycemic diabetic rats were treated with NO-impregnated wound dressings. Wound healing was impaired in both hyperglycemic and normoglycemic diabetic rats compared with controls. NO restored healing rates in normoglycemic diabetic rats compared with controls after 10 days, and granulation and scar tissue was thicker in the NO-treated mice than in control mice. This thickness of granulation and scar tissue was greater in the mice receiving the higher doses (Bohl Masters, Leibovich, Belem, West, & Poole-Warren, 2002). Together these studies indicate that NO improved both rate and quality of healing.

Topical GTN has been used successfully by de Berrazueta et al. (1994) in short term prophylactic treatment of post surgical phlebitis, very significantly \((p < 0.001)\) reducing both inflammation and pain post sclerotherapy for varicose veins. Notably, no compression bandages or stockings were used in this study. Topical GTN was shown to reduce inflammation within hours, compared with placebo.
3.3.5.11.4 Tendinopathy

The Level II evidence (NHMRC category)\(^2\) for GTN therapy in tendinopathy provides useful detail regarding effective small doses on the extremities (Paoloni, Appleyard, Nelson, & Murrell, 2005; Paoloni & Murrell, 2007; Paoloni, Murrell, Burch, & Ang, 2009).

Tendinopathy is a condition where little or no inflammation is present (Andres & Murrell, 2008; Khan, Cook, Bonar, Harcourt, & Astrom, 1999). This lack of inflammation was demonstrated histopathologically, suggesting a rethink and update regarding the implications for clinical management of tendinopathy from traditional methods focused on reducing inflammation. This was supported in reviews by both groups, Andres and Murrell (2008) and Khan et al. (1999). Histopathologic changes in tendinopathy include degeneration and disorganisation of collagen fibers, increased cellularity, and minimal inflammation (Andres & Murrell, 2008). The action of GTN on collagen reorganisation may be the mechanism of positive effects on tendinopathy.

A study using GTN for supraspinatus tendonitis showed improvement in pain at very early time points of 1, 2, and 15 days after 3 days of treatment with GTN patches (de Berrazueta et al., 2003). These results suggest a combined treatment effect of analgesia in the short term, as well as promotion of healing in the longer term, when GTN is used in tendinopathy.

3.3.6 Glyceryl trinitrate prescribing information

Although some forms of low dose GTN are available over the counter without prescription, GTN transdermal patches are an S4 drug and therefore require a medical doctor’s prescription. Some relevant considerations to prescribing GTN are presented here, but full information is supplied in the detailed full product information available in MIMS and from the manufacturing drug companies prescribing information (Australian Medicines Handbook 2015).

\(^2\) Examples of NHMRC levels: Level I systematic review of Level II studies; Level II randomised controlled trials; Level III experimental studies with or without a control group, quasi-experimental study; and Level IV case series (National Health and Medical Research Council, 2009)
3.3.6.1 Contraindications

The following contraindications to GTN patch therapy exist:

- Hypersensitivity or intolerance to organic nitrate compounds, or known or suspected sensitivity to any components of the patch elements
- Acute circulatory failure associated with marked hypotension (shock, states of collapse)
- Marked anaemia
- Conditions associated with increased intracranial pressure, including increased intraocular pressure, e.g., glaucoma
- Myocardial insufficiency due to obstruction such as in the presence of aortic or mitral stenosis, constrictive pericarditis, or hypertrophic cardiomyopathy
- Hypotension
- Allergy: Some literature exists about allergic reactions to GTN patch therapy. However, serious reactions are reported as being uncommon, and reactions are generally a low grade local hypersensitivity. In a study of 320 subjects aimed at determining the incidence of allergic reactions to GTN patches, 21 subjects (6.5%) had some reaction to the patches, 17 (5.3%) had a local irritant reaction, four patients (1.2%) had both local and remote effects, and one patient had an anaphalactic reaction (Kounis et al., 1996). Local reactions were reduced by changing to an alternative patch system, suggesting sensitivity to patch components other than the active GTN agent (Kounis et al., 1996; Merck Sharpe & Dohme Corp., 2014).

3.3.6.2 Drug interactions

Interactions of GTN occur with other drugs including warfarin, alcohol, and drugs used to treat hypertension, heart failure, and erectile dysfunction, e.g., sildenafil (Viagra). Concomitant use of GTN and phosphodiesterase type 5 inhibitors such as sildenafil are contraindicated because these drugs may amplify the vasodilatory effects of GTN and result in severe hypotension. A full list of known interactions is detailed in MIMS online (MIMS Australia, 2015).
3.3.6.3 Precautions

Caution should be used with concomitant use of GTN and alcohol, and in conditions associated with the risk of hypotension such as unstable hypertension, anaemia, and other conditions in which there is a potential risk of hypotension (Merck Sharpe & Dohme Corp., 2014).

Concomitant treatment with vasodilators, antihypertensives (calcium antagonists, beta blockers, ace inhibitors, and diuretics) tricyclic antidepressants, major tranquillisers, anticonvulsants, and dihydroergotamine may potentiate the blood pressure lowering effects of GTN. Therefore, adjustment of dosage may be required in these circumstances (Merck Sharpe & Dohme Corp., 2014).

Dose accumulation can occur if other nitrates such as Rectogesic, McGloin’s chilblain cream, sublingual spray, or tablets are used simultaneously with transdermal GTN patches.

Safety and efficacy of transdermal GTN in cases of venous ulceration are not established. There is a preferential dilation of venous vessels over arterial vessels (Merck Sharpe & Dohme Corp., 2014) which could increase venous incompetence, potentially exacerbating venous wounds.

Determination of the balance between risks and benefits in cases with venous incompetence should be considered only with careful monitoring on an individual basis until further research illuminates this point.

While literature exists citing the use of GTN in novel and wide ranging applications, has been reviewed in this chapter, specific indications, definitive dosages, and ideal regimens are not yet known for therapeutic applications for pedal ischemia due to the preliminary stage of research in this area.

Although no literature exists regarding any link with GTN and pathology, vigilance with temperature monitoring for hot spots as a preventative practice to predict and prevent breakdown is established as best practice in high risk feet as per the recommendations of
Armstrong et al. (2007) and should also be used to monitor any local vasodilatory effects of GTN on temperature change in high risk and neuropathic cases. Raised temperature sites greater than 4°C relative to the opposite foot have been identified as an indicator of incipient ulcer sites and as indicators for the development of Charcot’s neuroarthropathy\(^3\) (Lavery & Armstrong, 2007). Although the mechanism of vasodilation from GTN is physiologically distinct between this and the inflammatory processes of the conditions above, any possible and as yet unexplored potential risk should be taken into account and proactively managed within appropriate research protocol for GTN in high risk feet.

### 3.3.6.4 Side effects

Side effects are dose related. Adverse reactions most commonly include headache and, less often, dizziness, facial flushing, nausea, vomiting, and contact dermatitis. Cardiac disorders are rare. Investigations are indicated for heart rate increases (Merck Sharpe & Dohme Corp., 2014).

If any adverse drug related outcomes are experienced, immediate removal of the patch is advised and this initiates reversal of its effect. The drug is eliminated within two hours of patch removal. Patients who develop unstable blood pressure or hypotension should seek medical advice and may be withdrawn from GTN use (Merck Sharpe & Dohme Corp., 2014). Headaches are the most common reason for withdrawal from studies involving GTN (Santoro, Rovati, Setnikar, Caplain, & Gualano, 2000).

### 3.3.6.5 Temperature effects

As well as the documented floor on measurable toe pressures in cold conditions when skin temperatures are < 20 °C, conversely, warm conditions are known to enhance vascular supply to pedal extremities. GTN is known to have markedly increased effects when skin is well perfused, and both skin and ambient temperatures have been linked with significantly increased absorption (Barkve et al., 1986; Coakley, 1983; Klemsdal, et al., 1992). In a study

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\(^3\)Charcot’s neuroarthropathy is a disorder of the neuropathic foot, whereby structural collapse, usually of the midfoot, occurs after the sequelae of injury from either micro or macrotrauma that has been neglected due to the absence of protective pain sensation. Inflammation, hyperaemia, then structural damage ensue. Amputation may be required to manage the severe foot deformity. Strict offloading and stabilisation is needed. Early intervention is critical to outcomes.
investigating GTN absorption associated with high temperatures, Barkve et al. (1986) found that plasma concentrations on the control conditions were from 1.0 nmol/L ($SD \pm 0.8$ nmol/L) to 1.5 nmol/L ($SD \pm 1.0$ nmol/L). In the same study, on a different day when participants spent 20 minutes in a sauna, GTN concentration in plasma rose more by nearly fivefold to 7.3 nmol/L ($SD \pm 1.7$ nmol/L).

In two equine experiments using GTN for laminitis in mid-range temperature environments, positive effects of vasodilation and relief from laminitis occurred (Hinkley, Fearn, Howard, & Henderson, 1996; Simon, Bailey, & Elliot, 2004). Conversely, in a third equine experiment for laminitis, the researchers found that GTN had no effect on distal perfusion (Hoff, Hood, & Wagner, 2002). However, they stood the horses in iced water, then applied GTN, hypothesising that a faster rate of warming from the cold state would be associated with GTN treatment. The hypotheses about warming and effectiveness of GTN was disconfirmed in the induced cold state. This adds to the information demonstrating that GTN is most effective when perfusion is maximised, and its effect might be ameliorated or negligible in cold extremities and/or in severe ischemia.

### 3.3.7 Summary of literature on GTN

The body of literature that exists for the well-established uses of GTN lends weight to arguments supporting its efficacy, safety, and reliability. The few studies about the effects of GTN on pedal perfusion and wound healing include some older case studies reporting dramatic results that suggest value in further research.

In contrast to the wide range of applications of GTN and its established place as the mainstay of angina management, there is a paucity of research regarding the use of GTN in conditions of the vascularity compromised foot. This literature review reveals that there is much more yet to be discovered about the role of transdermal GTN and pedal perfusion.

From its first public experimental beginnings where vasodilation was demonstrated in a frog’s foot, GTN therapy showed promise as a therapeutic agent to assist in ameliorating the effects of impaired pedal circulation. However, only a small amount of evidence of
variable quality was found in the existing literature for the use of GTN specifically on the periphery, and even less for ulcer prophylaxis and healing in chronic foot wounds. The body of background evidence for the effective use of GTN presented in this literature review strongly supports the case for further research of this agent for podiatric applications. In particular, its applications in combatting pedal ischemia warrant exhaustive exploration.

Much of the burden of foot morbidity is preventable with optimal podiatric and multidisciplinary care (Mills, Armstrong, & Andros, 2010; Rogers, Lavery, & Armstrong, 2008; Rogers et al., 2010; Isenberg, Ridnour, Espey, Wink, & Roberts, 2005; Petrova & Edmonds, 2006, Ogrin 2013). It has been previously identified that additional benefits in the areas of high-risk foot care may be gained with advances in bioactive compounds such as nitric oxide donors (Isenberg et al., 2005; Petrova & Edmonds, 2006).

The evolution of topical GTN from ointments to transdermal patches has enabled large gains in the accuracy of application of specific dosage. Correct dosage is a critical element to effectiveness. The body of work over several years pioneered by Paoloni clarifies in detail the small dose ranges effective for some varying types of tendinopathy. Rayman et al., (2003) mention dose titration for determination of effective doses to overcome the issue of large individual variations in absorption in the periphery as documented by Francis et al., (1977). Dose titration to effect is standard practice for the use of GTN in Rectogesic for haemorrhoids and anal fissure. This topic area is ripe for development and the promise of discovery of the key factors in efficacious application of GTN is evident in the wide base of literature demonstrating its effectiveness in such varied conditions and applications.

The authors of two older case study collections outlined in Section 3.5.11.1 (Lund, 1948; Wheeland, 1983) describe remarkably positive outcomes in advanced cases of PAD with some complex co morbidities. Both of these authors recommend that further research be done to follow up on the apparent positive results. However, the quality and rigour of this apparently spectacular evidence is low.

In addition to the potential value of GTN in vascular perfusion, the prospects of effective analgesia that is safe and low cost, particularly in the prevalent and life-affecting problem
areas of ischemic and neuropathic pain, deserves thorough exploration and prompt application of any benefits.

3.4. Assessment modalities and considerations.

The tests initially envisaged for inclusion in both the pilot and the main experiment are all described here with their literature base and some considerations for their applicability in this context.

3.4.1 Temperature measurement

3.4.1.1 Ambient temperature

Close links have been demonstrated between ambient temperatures and their effect on peripheral vascular supply. Authors consistently recommend stable, temperate ambient conditions for accurate measurement of peripheral vascular status, with minimums between 21 and 24 °C (Bonham, 2011) and an ideal range of 24.5 ± 0.5 °C (Høyer, Sandermann, & Petersen, 2013a). Accurate ambient temperatures can easily be obtained from standard, well-calibrated thermometers, and these were used in the pilot study.

3.4.1.2 Skin temperature

When the skin is cold, peripheral blood vessels constrict, aiding corporeal thermal homeostasis. This vasoconstriction confounds attempts at accurately measuring peripheral vascular perfusion. The effects of arterial disease can be separated from this vasoconstriction only when the skin temperature is raised above 19 °C (Sawka & Carter, 1992). As a result of this, it is essential to assess skin temperatures as a precursor to valid assessment of toe pressures. Highly accurate skin temperature thermometers such as the Exergen are designed for this purpose and were used in the pilot study.
3.4.2 Vascular assessment

Accurate vascular measurement of the most distal parts of the foot is necessary in the quantification of therapeutic effects of GTN. Accurate toe pressures have only recently become available with technology outside the vascular laboratory.

Several issues relating to sensitivity and specificity of vascular assessment have relevance to the methodological considerations. Clinical tests with diagnostic accuracy from high sensitivity and specificity in detecting PAD have significant advantages that enable the most appropriate clinical decision making and referrals in that they avoid the costs and time lags intrinsic to laboratory testing. Table 4.1 provides information about the sensitivity and specificity of several clinical methods for peripheral vascular assessment. Among these, traditional visual vascular screening such as skin colour, skin quality, absence or
### Table 3.3

**Sensitivity and Specificity of Clinical Vascular Measurements for Detection of PAD**

<table>
<thead>
<tr>
<th>Clinical tests</th>
<th>Test considerations/conditions</th>
<th>Sensitivity/specificity for DDX of PAD</th>
<th>References</th>
</tr>
</thead>
</table>
| Visual assessments  | Pale, red, or blue limb  
Absent or reduced hair  
Atrophic skin          | 35/87%  
24–35%/84–87%  
"Poor sign" 43–50%/70–71% | McGee and Boyko (1998)  
McGee and Boyko (1998)  
McGee and Boyko (1998) |
| Cold limb           | Unilateral cooler skin                                                                         | 10/98%                                | McGee and Boyko (1998) |
| Buerger’s sign      | Pallor on elevation, rubor on dependency, indicative of vasomotor failure                    | 100/54%                               | McGee and Boyko (1998) |
| ABI                 | In general population  
In high risk populations (elderly, renal disease diabetes)  
In haemodyalysis      | 83–99/ up to 79%  
Sensitivity down to 15%  
Sensitivity 29.9%    | Xu et al. (2010)  
Xu et al. (2010)  
Okamoto et al. (2006) |
| Abnormal pedal pulses| DP and PT pulses both absent or one present and one weak. Pulses known to be clinician-dependent with false positives and false negatives. | 63–95%/73–99%  
81.93%/53–70%       | McGee and Boyko (1998)  
Williams et al. (2005) |
| Doppler sound and waveform evaluation | Control  
Non neuropathic diabetes  
Neuropathic diabetes | 86/96%  
100/92%  
94/66% | Williams et al. (2005)  
Williams et al. (2005)  
Williams et al. (2005) |
| TBI–with PPG technology | Automated twin cuff TBI compared with laboratory vascular test  
Detection of PAD compared with laboratory vascular test  
In haemodyalysis  
In diabetic gangrene  
ABI < 1.4  
ABI > 1.3  
Nondiabetic controls  
Diabetes  
Known PAD | 98.8/61%  
79/95%  
91.7/86.7%  
Approx. 80/80%  
92% sensitivity  
99% sensitivity  
100/81%  
91/65%  
90% sensitivity | Høyer et al. (2013b)  
Bonham et al. (2010)  
Okamoto et al. (2006)  
Park et al. (2012)  
Weinberg (2012 as cited in Høyer et al., 2013b)  
Suominen (2008 as cited in Høyer et al., 2013b)  
Williams (2005 as cited in Høyer et al., 2013b)  
Williams (2005 as cited in Høyer et al., 2013b)  
Williams (2005 as cited in Høyer et al., 2013b)  
Carter (1971 as cited in Høyer et al., 2013b)  
Carter (1971 as cited in Høyer et al., 2013b) |
reduction of hair, nail changes, and skin temperature, while widely used for screening, are shown to be low in sensitivity with the exception of Buerger’s sign\(^4\) which is present only in severe PAD. These methods were therefore not used in the research reported in this thesis.

The ankle brachial index (ABI\(^5\)) is widely relied on because it has strong links with cardiovascular disease risk and, as indicated in Table 4.1, it is a sensitive and specific test of vascular assessment in the general population. However, its sensitivity is so low (15%) in populations at risk of pathology related to pedal vascular insufficiency (diabetes, nephropathy, and the elderly) that its diagnostic value is seriously limited in these high risk populations.

Assessment of pedal pulses is associated with relatively high levels of both sensitivity and specificity. The two most sensitive and specific measures of clinical vascular assessment are Doppler ultrasound evaluation, (which provides a broad indication of vascular supply) and TBIs (which can give an indication related to ulceration and amputation prognosis (Sonter, Ho, & Chuter 2014). All three of these methods for determining pedal vascular supply were therefore used in the pilot study. More information about each of them, as well as about brachial blood pressure due to its role in determining TBIs, is provided immediately below.

### 3.4.2.1 Pulses

The sensitivity and specificity of pulses in vascular assessment is reported with various values from different authors. Williams et al. (2005) found that pulses are less reliable in the presence of diabetes and neuropathy. In people without diabetes, pulses provide 81% sensitivity and a high specificity of 93% for the detection of PAD. This is in agreement with the findings of other researchers (McGee & Boyko, 1998). In people with diabetes and neuropathy, however, these levels fall to 53% and 70% respectively (see Table 4.1).

---

\(^4\) Buerger’s sign is pallor on elevation of an extremity and rubor on dependency, indicating severe PAD. The redness occurs when hypoxia of the tissues triggers permanent maximal dilation of the peripheral vessels.

\(^5\) ABI and ankle brachial pressure index (ABPI) are synonyms referring to the systolic blood pressures at the ankle and brachium expressed as a ratio. This gives an indication of the peripheral ankle pressure relative to the systemic BP.
Despite the issues with pulse sensitivity and specificity associated with pathology, pulses were included as a measure in this study because the absence of pulse is an important sign and may indicate severe PAD in the stenosis or occlusion of a vessel.

Three pulses are normally palpable at three locations in the human foot: the dorsalis pedis pulse found on the dorsum; the tibialis posterior pulse, inferior to the medial malleolus; and the tibialis anterior pulse, a more proximal feeder to the dorsalis pedis artery, located centrally between the two ankle maleolli on the anterior ankle joint. The dorsalis pedis pulse is anatomically absent in about 6.7% of people (Yamada et al., 1993). The tibialis posterior pulse is often impalpable due to sub malleolar oedema common at this site. For this combination of reasons, and its ease of location, the tibialis anterior pulse was chosen for assessment in the pilot study.

### 3.4.2.2 Doppler ultrasound arterial evaluation

As shown in Table 4.1, Doppler sounds and waveform representations of arterial supply are have good to excellent sensitivity and specificity for clinical vascular assessment, particularly in the case of PAD and other conditions that are known to make vascular assessment less reliable (Williams et al., 2005).

Doppler technology uses a sensitive transducer that reflects ultrasound waves from circulating blood cells and gives a sound and a waveform reading that are both accurate indications of distal blood supply. This method was adopted in the pilot study due to its accuracy and acceptance in clinical vascular assessment.

### 3.4.2.3 Brachial blood pressure

Assessment of brachial blood pressures (BPs) is essential for determining TBIs. Brachial BPs provide an indication of the systemic blood pressure. However, they can be distorted by a number of conditions. These are listed, with their effects, in Table 4.2. The effects of these conditions on brachial blood pressure underscore the importance of consistency in measurement protocol (Welch Allyn Inc., 2010).
BPs were taken in the study with validated automated desktop sphygmomanometers as the necessary precursor to TBIs, the primary outcome measure of the study.

Table 3.4

*Effects of Measurement Conditions on Brachial Blood Pressure Readings*

<table>
<thead>
<tr>
<th>Activity/element</th>
<th>Effect on systolic pressure in mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cuff too small</td>
<td>10–40↑</td>
</tr>
<tr>
<td>Cuff over clothing</td>
<td>10–40↑</td>
</tr>
<tr>
<td>Back / feet unsupported</td>
<td>5–10↑</td>
</tr>
<tr>
<td>Legs crossed</td>
<td>5–8↑</td>
</tr>
<tr>
<td>Patient talking</td>
<td>10–15↑</td>
</tr>
<tr>
<td>Laboured breathing</td>
<td>5–8↑</td>
</tr>
<tr>
<td>Full bladder</td>
<td>10–15↑</td>
</tr>
<tr>
<td>Pain</td>
<td>10–30↑</td>
</tr>
<tr>
<td>Arm below heart more than 46mm</td>
<td>↑</td>
</tr>
<tr>
<td>Arm above heart more than 46mm</td>
<td>↓</td>
</tr>
</tbody>
</table>


(This table is sourced from 51 citations, from 20 peer reviewed journal articles).

### 3.4.2.4 ABI versus TBI

In their review of toe pressure measurements for diagnosis of arterial disease, Høyer et al. (2013b) state that this area is in a preliminary state of development. Further work providing a bank of reliable normative values both with and without pathology has been recognised as important. However, toe pressures are already recommended by international guidelines for comparative and additional value as part of a vascular exam. “Regardless of the ambiguous definition of diagnostic limits, there is an interesting correlation between the TBI and comorbidities, such as kidney disease and diabetes, which suggests that the TBI in some aspects, is more valid than the ABI” (Høyer et al., 2013b, p. 236).

Hyun et al. (2014) report a distinction between cardiovascular mortality predicted with ABI and TBI in people with and without diabetes. The relationship of CVD mortality in diabetic individuals with clinically suspected PAD indicates increased risk with ABIs that
extend beyond both the high and low thresholds of normal. In contrast, for people without diabetes, a linear relationship with cardiovascular mortality exists between both ABIs and TBIs, with lower values predicting higher risk and higher values predicting lower risk. This indicates that the TBI performs better than ABI in flagging CVD risk due to the reliable linear relationship between values and risk status.

Craike et al. (2013) report that sensitivity of TBIs is 95% compared with 47% for ABIs in the detection of PAD. Hyun et al. (2014) agree about the superiority of TBIs over ABIs. However, other authors (Brookes et al., 2001; Stoenbroek, Ubbink, Reekers, & Koelemay, 2015) have reported that TBIs are not of added value to ABIs in vascular assessment. Further work to extend understanding in this area is warranted. Clarification about study populations, in particular the presence of diabetes which has been shown to influence the validity of these tests differently, and examination of details of test protocol including cuff size, may shed light on the reasons for these conflicting results.

3.4.2.5 Absolute toe pressures versus toe pressure indices

A distinction exists between absolute toe pressure (TP) and its related index, the TBI. Toe Pressure is a simpler measure of the vascular systolic pressure in the toes alone, whereas the TBI is the systolic toe pressure divided by the systolic brachial blood pressure. People with systemic hypertension may have higher TPs that do not reflect relatively greater distal perfusion (Høyer, Sanderman, and Petersen, 2013b).

Theoretically, due to the inclusion of brachial pressures as an indicator of systemic blood pressure in the calculation, TBIs should provide a more valid measure of ischaemia in the periphery than do TPs. However, the brachial vessels and their proximal feeder arteries can also contain sclerotic changes which can confound the measurement of systemic pressure, and this is more likely in advanced disease. Sonter, Ho & Chuter (2014) in a recent systematic review and meta-analysis about both TPs and TBIs, were not able to draw a distinction between the validity of TPs versus TBIs. They found that the limited evidence available supports an association of both low TPs and low TBIs with risks of nonhealing and amputation.
The single measure of absolute toe pressure (TP) is most often used in the literature in discussion of low end pressures to assist with surgical options, including the determination of amputation levels (Caruana, Formosa & Cassar 2015, Sonter et al. 2014, Varatharajan et al. 2006). The TBI in contrast, is more often referred to in the literature in regards to the upper level measures; in screening for the diagnostic threshold levels for PAD (Høyer, Sandermann & Petersen. 2013b).

The issue of the additional complexity versus the accuracy of including brachial pressures is worthy of further research attention, as insufficient evidence currently exists to make a distinction of the value between these two measures (Sonter, Ho & Chuter 2014). This reflects the preliminary state of the literature available in this area. These authors recommend that, whenever possible, it is best practice to include brachial pressure measurements in the clinical picture to give the most complete assessment of perfusion of the foot relative to the systemic pressure.

3.4.2.6 Toe brachial indexes

In comparing clinical and laboratory measures for vascular assessment, TBIs fulfil the need for accurate and clinically accessible, quantitative vascular measurement of distal foot perfusion. Accurate TBI technology has very recently evolved from being available only in vascular laboratories to being available as portable, affordable technology in clinical settings. Improvements in TBI accuracy have been achieved by using a combination of photoplethysmography (PPG) as the sensing method, twin cuff technology, and automated versus manual cuff inflation.

Portable TBI units using PPG were previously available only in manual units with poor reliability (Romanos, Rasopvic, & Perrin, 2010) or as a laboratory test. Bonham, Kelechi, Mueller, and Robison (2010), Høyer et al. (2013a), and Perez-Martin et al. (2010) have recently compared the accuracy of automated portable TP and TBI testing with laboratory methods, and have all found the clinical TBI assessment to be comparable in accuracy with laboratory methods. The latter two of these researcher groups used the automated Systoe
device (Atys Medical, France), as used in both the pilot study and the main experiment reported in this thesis.

Using this device, toe pressure is procured in a similar manner to brachial pressure, but the sensor needs to be additionally sensitive to detect the more subtle signals of supply in the periphery, and the measurement is more subject to variation. A sensor to read the blood supply is attached to the end of the toe, and a more proximal occlusion cuff is inflated until loss of signal. The occlusion cuff is gradually released and the pressure at which the signal returns is recorded as the TP.

There is a lack of information specific to TBIs about the conditions that influence brachial blood pressure that are listed in Table 4.2. Because of this, extrapolation of the findings in that table to TBIs seems reasonable. Furthermore, clinical experience provides evidence that perturbation such as test subjects’ coughing, talking, sneezing, and moving cause temporary notable increases in TBIs.

Toe pressures are subject to a number of influences that need to be taken into account but have not been completely explored in the literature. The influences that appear to be most important are identified below.

3.4.2.6.1 Supine level positioning

It appears necessary to take measurements of toe or ankle pressures in a flat supine position with the heart and feet at the same level. This avoids error due to hydrostatic pressure of the column of blood over the extremity (Carter, 1993). Elevation of the torso significantly elevates TP (Gornick et al., 2008), and leg elevation or dependency significantly affects TP (Wiger & Styf, 1998). It is, however, problematic for some people to lie flat due to medical conditions such as respiratory and musculoskeletal disorders. Gornick et al. (2008) provide an algorithm to accurately calculate the ABI for these people, which takes into account the vertical distance of the ankle cuff from the heart. This work has been validated, but no similar work exists for toe pressures.

3.4.2.6.2 Pre-test rest times
Pre-test rest times can affect the outcomes of brachial, ankle, and toe pressure readings (Sadler, Chuter, & Hawke 2014). Recommendations vary from 6 to 30 minutes of rest time needed for accuracy of these measures, and national guidelines do not provide definitive recommendations in this regard.

Given that lack of time was the primary reason given in studies for not conducting ABIs in practice, Sadler et al. (2014) sought to improve both the accuracy and uptake of the use of TP technology by authoritatively stating the minimum rest times needed for measurement accuracy.

A decline in brachial blood pressure has been noted for the initial 10 minutes of sitting or lying (Sadler et al., 2014). In their systematic review to determine the effect of pre-test duration on toe and ankle systolic blood pressure measurements, Sadler et al. (2014) found no evidence of the effect of rest times, but this was due to the lack of any research that fulfilled their selection criteria. They therefore highlighted the need for rigorous studies in this area.

3.4.2.6.3 Skin and ambient temperatures

Skin temperature is a measure typically included in the clinical vascular assessment. Although low skin temperature has low sensitivity (10%) but high specificity (98%) for the diagnosis of PAD.

Researchers relating skin temperature to distal lower limb blood pressure measurements are in agreement that TP measurements are unreliable and usually unprocurable when skin temperatures are below 20 °C (Bonham, 2011; Cloete et al., 2009; Høyer et al., 2013b; Sawka & Carter, 1992), and that warming is necessary. There is no consensus, however, about the method by which warming is best achieved. Høyer et al. (2013b) have highlighted this inconsistency and recommended further research to clarify this point. In their review they reported that the presence or absence of warming made consistent differences to both toe pressure measurements and ranges of normal values. Sawka and Carter (1992) investigated the effects of temperature on digital pressures using three differing protocols. For 77 limbs with arteriosclerosis obliterans, they confirmed that low skin and ambient
temperatures create unreliable testing conditions and proposed that routine testing involve covering the feet with a “heating blanket” for at least 20 minutes.

3.4.2.6.4 Cuff sizes

Cuff size for digital pressure measurements is an important parameter according to the only authors publishing specifically about this topic (Påhlsson, Jorneskog, & Wahlberg, 2004; Påhlsson et al., 2007), who state that it is ideal to use a cuff of 2.5 mm whenever this size will fit the toe. This sized cuff gives the most accurate measurement (Påhlsson et al., 2007). Smaller cuffs exert more variable and higher pressures on the arteries under examination, delivering some lower but usually significantly higher TP values. The increases associated with small cuffs varied widely between 2 mm Hg and 27 mm Hg in a study of 20 people (Påhlsson et al., 2007). Due to this small sample size this evidence is very low level.

3.4.2.6.5 Vascular hyperaemia and repeat measurements

Vascular hyperaemia is a phenomenon noted in normal extremities after release of a compression or restriction of flow. A relative vasodilation occurs following compression, creating an increase in vascular pressure. This flush of increased vascular supply can be a regulating and useful phenomenon in the measurement of digital pressures as it can overcome some of the constriction that may be present due to cold temperature or lower vasomotor tone and create a pressure representative of maximal supply. Typical supply would be expected to be less than maximal supply unless Buerger’s sign is present (indicating maximal vasodilation), or as a vascular complication of ischaemia and diabetes where the mechanisms of homeostatic adjustment of vasomotor tone of the distal foot are attenuated. Because of this, Perez-Martin et al. (2010) suggest performing three measures of TBI, discarding the first because it is likely to be misleadingly low, and averaging the second and third.
3.4.3 Neurological assessment

Neurological impairment is associated with diabetes, vascular compromise, and ageing, and may involve changes that can affect motor and sensory systems in various patterns depending on the specific pathology. A combination of assessment measures is recommended to give a full neurological picture of the status of function and the location of neurological deficits (Smieja et al., 1999).

Six neurological assessment domains were considered for the pilot study. The reported sensitivity and specificity for each of them are provided in Table 4.3.

### Table 3.5

<table>
<thead>
<tr>
<th>Neurological element</th>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain</td>
<td>1. History</td>
<td>NA(^d)</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>2. Visual analogue pain score</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Protective sensation(^a)</td>
<td>10 gm monofilament</td>
<td>77%–88%</td>
<td>68%–96%</td>
</tr>
<tr>
<td>Vibration sense(^b)</td>
<td>Tuning fork</td>
<td>96%</td>
<td>46%</td>
</tr>
<tr>
<td>Position sense(^c)</td>
<td>Position sense test</td>
<td>20%</td>
<td>98%</td>
</tr>
<tr>
<td>Temperature discrimination</td>
<td>Test tubes</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Balance</td>
<td>Romberg’s test</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

\(^a\) (Tan & Tan, 2010)
\(^b\) (Shy et al., 2003a)
\(^c\) (Smieja et al., 1999)
\(^d\) NA indicates either that sensitivity and specificity are not applicable for this test or that no validated information exists in these domains.

3.4.3.1 Pain

Pain history is difficult to determine retrospectively as memory and perception in this highly subjective area vary and fade over time. Tesfaye et al. (2010) state that neuropathic pain can be reliably assessed with a visual analogue pain scale (VAPS). This has been
widely used and validated for this purpose. The VAPS is a valid and reliable method to assess and monitor both acute and chronic pain (Bijur, Silver, & Gallagher, 2001).

The VAPS involves the subject aligning a sliding marker on a double sided ruler with the numbers from 0–10 on one side intended to reflect the relative intensity of a specific pain experience. On the other side of the ruler, there are five faces with expressions representing a range from very distressed and crying, to happy. This gives an alternative way of rating the pain experience that can be readily requested of the test subject, and then quantified.

In the pilot study, both of the above ways of assessing subjects’ experience of pain were used. First, semi-structured verbal questioning was employed. This was subsequently replaced with the VAPS.

### 3.4.3.2 Protective sensation

Protective sensation is an indicator of intact sensory nerve function and its absence is the single most predictive risk factor for ulceration (Boulton et al., 2008). Accurate measurement of protective sensation is therefore highly desirable. The Semmes-Weinstein monofilament examination, a reproducible, valid, and generalisable test of foot sensation, is recommended as a screening procedure for examining diabetic feet by international guidelines and systematic reviews (Boulton, Malik, Sosenko, & Arezzo, 2004; Smieja et al., 1999). The monofilament is a simple, hand-held tool consisting of a synthetic flexible filament of accurately graduated thickness that buckles at a calibrated application force. The level of this buckling force in the 10 gm monofilament corresponds with a threshold for protective sensation.

The test is performed by placing the monofilament on each of 10 sites on each foot and holding it for at least one second, asking subjects to verbally respond when they can feel the stimulus. A score is given for each responsive site. The Carville protocol states that a score < 6 indicates loss of protective sensation (LOPS). Other protocols use fewer sites and state that lack of response at any site indicates LOPS (Tan & Tan, 2010).
Simejia et al. (1999) compared the reproducibility and accuracy of other standard neurological and vascular clinical examinations with monofilament examination for the determination of protective sensation. All other conventional clinical neurological examinations had lower reproducibility and accuracy compared with the monofilament. Therefore it was used as the method for determining protective sensation in the pilot study.

### 3.4.3.3 Vibration sense

Vibration sense is an indicator of large nerve fibre function. This is related to pressure perception and is therefore clinically associated with foot ulceration, falls risk, and loss of balance (Boulton et al., 2008). Vibration sense is normally reduced with ageing, significantly so by the eighth decade (Brocklehurst, Robertson, & James-Groom, 1982), by which time loss at the hallux is a normal occurrence (Verrillo, 1980). Although there has been a recent call for more accurate age-specific reference values for vibration threshold screening (Maffei et al., 2014), the Rydell Seiffer graduated tuning fork is regarded as an accurate tool for vibration testing, with 96% sensitivity and 46% specificity reported for the detection of neuropathy according to Kästenbauer, Sauseng, Brath, Abrahamian, & Irsigler (2004). Typically, the tuning fork is held to the hallux apex, alternating the vibrating and not vibrating status, and asking the subject to discern when the vibration is sensed. It was initially included in the pilot study due to its established use in sensory testing and the clinical relevance of the results.

### 3.4.3.4 Position sense

Position sense is a measure of proprioception, which is a clinically important measure of neurological function in the foot (Boulton et al., 2005). Diabetic sensorimotor polyneuropathy is a common form of neuropathy affecting about 50% of people with diabetes (Boulton et al., 2004), and can be seen in impairment of position sense and related neurological functions of vibration and light touch. Nerve function for position sense is anatomically conducted along the same spinal nerve tract as vibration sense and light touch. An impairment of any of these functions is often associated with loss of the other functions travelling in the same tract (New York University School of Medicine, 2006). Position sense is often determined by comparing passive alternating dorsi and plantar flexion positions and seeking indications of subjects’ awareness of the relative location of their toe.
Holding the great toe only on both sides and avoiding contact with adjacent toes and the toe nail are important elements that affect validity of this test. This test of proprioception was included in the pilot study for its clinical relevance.

### 3.4.3.5 Temperature discrimination

Temperature discrimination is lost with sensory neuropathy, carrying implications for increased risk of thermal injury (Claus, Hilz, Hummer, & Neundörfer, 1987). Shun et al. (2004) used nerve biopsies to demonstrate nerve fibre losses of concentrations related to temperature sensitivity that was reduced on average by 50% in people with diabetes. A test using warm and cool water in glass test tubes is a classic method for determining the presence of temperature discrimination in the foot. These test tubes are placed against the skin of the medial arch of the foot for the greatest contact area, with the client being asked to discriminate between the temperatures of each. Temperatures are typically neither standardised nor measured, except for the assessor’s own estimations of the degree of heating of the tap water for their subjects’ comfort and safety, and to allow distinction of the different temperatures. This is determined after alternating warm and cool test tubes until it is ascertained whether temperature discrimination is present or absent. Although this method is known to have many sources of uncontrolled variability, it was initially included in the pilot study because of its established place in educational podiatric practice.

### 3.4.3.6 Balance

Balance is related to proprioceptive function, which can be lost in advanced sensory neuropathy (Boulton et al., 2004). This is clinically related to neuropathic gait changes that are precursors of foot ulceration. Visual input from the vestibular system masks proprioceptive loss by supplementing its function, so subjects will lose balance when eyes are closed if proprioception is impaired. Romberg’s test evaluates the sensory input of the motor coordination of standing balance that depends on intact proprioception. The test involves observing subjects in a standing position after placing them safely near a support, with the tester being located to assist the subject in case of falling. Subjects are asked to close their eyes and the tester notes any evidence of postural sway that occurs with eyes closed for one minute. A notable extent of standing sway that increases with closed eyes is
abnormal. Romberg’s sign was initially used in the pilot study as a functional measure of proprioception.

3.5 Wound assessment

Several wound scoring systems are in common use for evaluating, monitoring, and reporting wound healing. The University of Texas and the Wagner scales are well known. They were compared by Oyibo et al. (2001). Neither of these systems differentiates between the severity of grades for ischaemia and infection, both of which are important clinical parameters when dealing with foot wounds in people with diabetes and other at-risk populations.

The Saint Elian Wound Score System (SEWSS) is a recently developed and validated tool that does include grading of both ischaemia and infection (Martinez-De Jesus, 2010). It produces a score out of 30 taken from three categories: anatomical locations, aggravating factors, and affected tissues. It includes 10 domains with scoring distinctions for each of the fields including anatomical location, four grades of infection, oedema, neuropathy, ischaemia, and three grades of wound locations, dimensions, and healing stages. The resulting score out of 30 is linked to validated prognosis for healing and a guide to the level of appropriate therapy (Martinez-De Jesus, 2010). This system was chosen for use in the pilot study over the other more well-known wound scoring systems due to its validated accuracy for assessment and monitoring of healing progress of diabetic foot ulcers. Appendix D contains the proforma for this wound scoring system.
PILOT STUDY

4.1 Introduction

The main purpose of this thesis is to determine whether improvement in distal vascular supply results from the use of GTN. That is investigated by means of an experiment in the next chapter. In this current chapter (Chapter 4), a number of preliminary methodological considerations were addressed in a pilot study. The following 13 clinical tests from four categories were used in the pilot:

1. Temperature measurement
   • Ambient temperature
   • Skin temperature

2. Vascular assessment
   • Palpation of tibialis anterior pulse
   • Doppler assessment of tibialis anterior pulse
   • Brachial blood pressure (BP)
   • Toe brachial indexes (TBIs)

3. Neurological testing
   • Pain
   • Protective sensation
   • Vibration sense
   • Position sense
   • Temperature discrimination
   • Balance

4. Wound analysis
   • Systematic wound scoring
The pilot study was conducted with twofold aims as follows:

1. To test accepted clinical neurovascular assessments, with a view to ascertaining their practicality for inclusion in a larger project on the effect of GTN on pedal ischaemia. Temperature, vascular, neurological, and wound score measurements were included.

2. To determine intra-tester reliability of student practitioners, using these measurements in a clinical context.

An unintended subsidiary outcome of this study involved assessing these measurements’ usefulness in clinical and teaching contexts.

This study was conducted at the Allied Health Clinic of Charles Sturt University, which became the Community Engagement and Wellness Centre halfway through the data collection phase of the study with changes of both the location and name of the university’s podiatry teaching clinic.

4.2 Method

Six subjects were assessed on two occasions, first by one half of a 24 student group who were moving from the third to final year of their podiatry course, then by the second half of that same student group. Temperature, vascular, and neurological testing were performed with these six subjects. An additional three subjects with wounds were recruited specifically to assist in the education regarding assessment of wounds.

The initial round of testing with the first cohort of students comprised 13 tests. In light of the results from that round, four tests were discontinued because of unsatisfactory reliability and/or validity, and one of those tests was substituted with an alternative. As a result, 10 tests were used with the second cohort of students.

4.2.1 Student participants

The podiatry student participants were recruited during their end-of-year podiatry clinic practicums. The group was split in half for two, 2-week clinic blocks. At this stage it was anticipated that students would assist as data collectors for the main experiment. Informed consent was obtained for their participation. See Appendix E.
4.2.2 Client participants

Client participants were assessed for their suitability based on the following inclusion criteria:

- Able to comprehend the issues of informed consent
- Able to communicate verbally in English
- Willing and able to lie flat for two 2-hour periods.

All of these people were existing clients of the podiatry service within the university’s clinic and were community dwelling, ambulant people aged between 69 and 78, comprising two females and four males. A range of age-typical impairments in their vascular and neurological status was present, having been established on previous podiatry treatment visits. All clients were given information about the study and were invited to provide their informed consent. See Appendix F.

4.2.3 Tests and instruments

4.2.3.1 Temperature measures

- Room temperature was taken from a digital desktop solar powered thermometer in each clinical cubicle.
- Skin temperature testing was performed with the validated Exergen skin temperature thermometer (Teran et al., 2012) at three sites: the apex of the hallux, the medial eminence of the first metatarsophalangeal joint, and over the dorsomedial eminence of the cuneionaviccular joint of the midfoot.

4.2.3.2 Vascular tests

Historically, pulse palpation has held a pivotal role in decision making in vascular assessment, and this is still widely taught and practised. It is recommended by the American Heart Association and in international guidelines for PAD diagnosis (Rooke et al. 2011, Norgren et al. 2007). It is non-invasive, quick, and inexpensive, and can contribute to first line examination impressions, building from visual and other palpation tests. Despite its limited sensitivity for PAD diagnosis, palpation of pulses is recommended in vascular...
assessment with a “strong” relative recommendation rating (Brownrigg, 2014). The evidence base is quoted as “weak” on the same (three point) scale. Despite this weakness, they are included along with a suite of tests to contribute to a body of clinical findings.

- Pulses of the tibialis anterior artery were digitally palpated and scored on a scale with reference points of zero for absent, 1 for a trace, 2 for normal, and 3 for a bounding pulse.

- For ultrasound Doppler testing, the hand-held portable 8 Mhz Hadeco Doppler model no. BT*Mo558C was used. Doppler readings were taken of the tibialis anterior pulse with scores on a scale from 0 to 3 with reference points of zero for no signal, 1 for a monophasic signal, 2 for a biphasic signal, and 3 for a triphasic signal.

- Brachial BPs were obtained with an Omron desktop sphygmomanometer, with only one reading taken on each subject’s left arm.

In light of the relevant literature on TBI influences, as presented in chapter 3. Section 3.4.2.6. and based on the most comprehensive best-practice protocol provided by Bonham (2011), the following decisions regarding TBI measurement were made:

- to rest the subjects in a supine lying position for 10 minutes prior to testing;
- to use the large 2.5 cm cuff by default, and the small one only when the large was too wide to accommodate both the measurement and sensor cuffs;
- to use warming only when toe pressures were unprocurable;
- to take three immediately consecutive measures of TBIs on each foot; and
- to avoid the subjects talking or other perturbations of the subjects during testing.

Appendix C specifies the protocol used for the pilot study. Additions regarding cuff size, as well as taking three rather than two TBI measures were made to the Bonham protocol.

- TBIs were taken three times of both great toes using one of three Systoe portable twin cuff automated PPG devices that were charged to maximum levels and checked for calibration before each session.
4.2.3.3 Neurological tests

- Pain was assessed by the first student cohort with a verbal history elicited via semi-structured questioning. In the second cohort, the VAPS ruler was used with the expectation that it would capture a more systematic, repeatable indication of pain history.

- Protective sensation testing was performed using the 10 g monofilament according to the Carville 10 site protocol (Smieja et al., 1999).

- Vibration testing with the graduated Rydel Seiffer 128 Hz tuning fork was performed by the first cohort only.

- Position sense was assessed according to the procedure described in Section 4.4.4. Appendix G contains additional information concerning this procedure that was provided to the students collecting the data.

- The temperature discrimination method described in Section 4.4.5 was used with the first cohort of students only.

- Romberg’s test, as described in Section 4.4.6, was used with the first cohort of students only.

4.2.3.4 Wound assessment

The Saint Elian Wound Score System was used according to the procedures described in Section 4.5.

4.2.4 Procedure

All students were prepared during an information session that involved education about the tests required including wound scoring and a data collector’s guide that provided procedural details and information about the way in which data were to be recorded. See Appendix G.
4.2.4.1 Temperature, vascular, and neurological measurements

Three podiatric clinicians, including myself as principal researcher, supervised the students as they performed the temperature, vascular, and neurological assessments and recorded the outcomes with the six subjects. Each of the subjects was tested in sequence, usually by six individual students, using the 13 or 10 assessment items of the study depending on the student cohort. Apart from student cohort-based differences in terms of number and nature of tests, each subject had all tests performed by every student, including three TBIs on each foot.

The sequence of these procedures was organised for optimal time management within the test sessions. First, the subjects were positioned lying flat, being offered two head pillows for their comfort. While they undertook their 10 minutes’ preparatory rest in recumbency, room and skin temperatures were recorded, then the neurological measures of pain assessment, monofilament, vibration (first cohort only), position sense, and temperature discrimination (first cohort only) were performed. The vascular measures were then obtained in the order of pulse palpation, Doppler pulse assessment, BP, and TBIs. No warming was necessary as the subjects’ foot temperatures were all > 20 °C. (This pilot study was conducted in summer when outside daily maximum temperatures were warm to hot, ranging up to 35 °C). The effects of peturbation and other influences were minimised by having the subjects lie still and quiet before and during testing. In the case of an unexpected perturbation such as sneezing or talking by the subject, the measurement was repeated. Romberg’s test was the last performed (first cohort only) because it would have unduly influenced the BP and TBI readings if performed earlier as it required the subject to stand.

4.2.4.2 Wound assessment

As part of the preparation of the students for the data collection role in the study, wound assessment was the topic of a tutorial involving three specifically recruited patients with chronic wounds. Students workshoped the wound scoring system, aiming at reliable categorisation of the wounds in the 10 domains of the SEWSS.

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1 The air conditioning thermostat for the clinic was set at 22 °C, and temperatures in the clinic were maintained between 21 and 24 °C for all testing episodes.
Supplementary education for the both cohorts was delivered with photographic images and scenarios involving group participation in rating cases of oedema, then the completion of an SEWWS proforma for a specific case.

4.2.5 Analyses

Given the small numbers involved in this study, it was possible to analyse most of the data satisfactorily by visual scanning. Apart from calculating means and determining modes and medians for some variables, statistical analyses were deemed unnecessary and inappropriate given the small amount of data involved in each set of comparisons. Data from the 13 tests were examined for any notable departures from means, modes, and medians.

4.3 Results

4.3.1 Temperature measurements

For both student cohorts, all recordings of room temperatures were within 2 °C of the mean of 22 °C. Skin temperatures at all three sites for the same individual were within 2 °C of each other. These results were regarded as being sufficiently similar to each other to indicate acceptable levels of reliability and validity.

4.3.2 Vascular testing

- Pulse measurements exhibited high variability across the four gradings (0–3) when students were assessing the same subject. For example, one student consistently showed a lesser ability to locate the pulses relative to the other students. Therefore the validity of pulse data when produced by students was regarded as questionable.

- There was a high level of agreement concerning the brachial blood pressure readings. These readings differed to only a minimal extent between students when based on the same subject, and some differences might have been caused by habituation of the participants to the repeated multiple measures in the trial. Even taking that possibility into account, the readings taken by the students were sufficiently similar to be regarded as reliable and valid.
• Doppler ratings differed to only a minimal extent between students when based on the same subject. Doppler ratings were done using a 4 point grading scale. There was no discrepancy greater than one point of difference in any measurement.

• TBI readings were also very similar when made by different students of the same subject. There were no significant differences between the TBI measurements made by each student. These were made with the validated desktop toe pressure device, the SysToe.

See Table 4.1 for a summary of the findings concerning consistency of each of the vascular tests within each cohort of students.

### Table 4.1

**Reliability of Vascular Tests**

<table>
<thead>
<tr>
<th>Vascular tests</th>
<th>Student Cohort 1</th>
<th>Student Cohort 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tibialis anterior pulse</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manual palpation</td>
<td>Inconsistent</td>
<td>Inconsistent</td>
</tr>
<tr>
<td>Doppler sound rating</td>
<td>Consistent</td>
<td>Consistent</td>
</tr>
<tr>
<td>Brachial blood pressure</td>
<td>Consistent</td>
<td>Consistent</td>
</tr>
<tr>
<td>Toe brachial index</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>Consistent</td>
<td>Consistent</td>
</tr>
<tr>
<td>Second</td>
<td>Consistent</td>
<td>Consistent</td>
</tr>
<tr>
<td>Third</td>
<td>Consistent</td>
<td>Consistent</td>
</tr>
</tbody>
</table>

### 4.3.3 Neurological testing

• Evaluations of subjects’ pain varied considerably for the same subject when pain history was determined by the first cohort of students, so history-taking was replaced by the VAPS ruler tool in the second cohort, in which there was a high level of agreement concerning the pain experience as recorded for the same subject by different students.

• Protective sensation results were consistent for both cohorts of students when the same subject was assessed by different students.
• The vibration test produced a marked intra-subject variation in the first cohort of students, so it was not used with the second cohort.

• Assessment of position sense provided moderate to good reliability. One student in Cohort 1 more frequently rated the position sense absent on 5 out of 12 (6 bilateral) rating possibilities. There was some variability in this result, particularly with two of the subjects who may have had attenuation of this sense as the accumulation of tests built up on each occasion. Most students found two of the 12 toes in the test to have absent position sense, which was consistent between raters.

• The temperature discrimination test produced very differing results among the first cohort of students, and was therefore not used with the second cohort.

• In Romberg’s test it was difficult for the students to quantify the amount of sway that was considered abnormal. The results reflected this uncertainty, with no consistent agreement between raters in the first cohort. Therefore this test was not used with the second cohort.

Table 4.2 contains a summary of the results of the neurological tests for the two cohorts of students.

Table 4.2

Reliability of Neurological Tests

<table>
<thead>
<tr>
<th>Neurological tests</th>
<th>Student Cohort 1</th>
<th>Student Cohort 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>verbal questioning</td>
<td>Inconsistent</td>
<td>NA(^a)</td>
</tr>
<tr>
<td>VAPS ruler tool</td>
<td>NA</td>
<td>Consistent</td>
</tr>
<tr>
<td>Protective sensation</td>
<td>Consistent</td>
<td>Consistent</td>
</tr>
<tr>
<td>Vibration</td>
<td>Inconsistent</td>
<td>NA</td>
</tr>
<tr>
<td>Position sense</td>
<td>Consistent</td>
<td>Consistent</td>
</tr>
<tr>
<td>Temperature discrimination</td>
<td>Inconsistent</td>
<td>NA</td>
</tr>
<tr>
<td>Romberg’s test</td>
<td>Inconsistent</td>
<td>NA</td>
</tr>
</tbody>
</table>

\(^a\) Not applicable.
4.3.4 Wound assessment

The use of the validated wound rating scale, the St Elian Wound score system (SEWSS) was introduced via a tutorial where students were shown a PowerPoint presentation of images of wounds displaying variations of the scoring elements. The student’s abilities to reliably rate several wound photographs and later the three wound participants were tested. In the initial cohort, the students were highly consistent and accurate in their scoring within nine of the 10 domains on the SEWSS, but they showed unacceptable inaccuracy and variability concerning odema, highlighting their lack of experience in the range of presentations of odema.

To remediate this problem, as noted in the procedure section above, specific additional information was incorporated in the second group’s educational preparation, resulting in a high level of agreement of all raters within one point of the total score out of 30.

4.4 Discussion and conclusions

4.4.1 Temperature measurements

Because the measurements of room temperature and skin temperature were reliable, these were retained unchanged for the major experiment.

4.4.2 Vascular measurements

- Digital palpation of pulses is known to give variable results, especially with inexperienced clinicians. It was decided, therefore, to retain the pulse measurement but with a single experienced data collector for the main experiment.
- Doppler rating was reliable, and was retained unchanged.
- The brachial BPs were also reliable and confirmed as a sound basis for the TBI calculations.
- TBIs were consistent within both cohorts of students and so were retained, being confirmed as suitable for the primary outcome measure in the larger study.
4.4.3 Neurological measurements

- The high variability of pain scores derived from the first cohort highlighted the difficulty of inexperienced raters in taking an accurate pain history. The VAPS ruler used in the second cohort provided consistent results. However, these more consistent results could have been related to the subjects remembering how they had responded to the VAPS only a short time (perhaps as little as 15 minutes) previously. The pain assessment was the only subjective test in the reliability trial. The VAPS ruler was carried forward for use in the major study, where in this context of single measures at each monthly visit, it was deemed to be appropriate and its advantages over a verbal history score could be tapped.

- Protective sensation (monofilament) testing was carried forward to the larger study due to the good reliability in this pilot, backed up by the excellent reputation of this test supported by the literature.

- The lack of reliability associated with the tuning fork test was not expected given its good reputation and widespread use. However, in their review of the graduated tuning fork, Kästenbauer et al. (2004) reported reduced accuracy for its use with inexperienced operators. Possible sources of error with the tuning fork included the variable force with which different operators set the fork vibrating, nonstandardised pressure used in applying it to the skin, and variation in the verbal instructions. No replacement for this test was used with the second cohort of students, but an alternative validated test for vibration (an electronic neurothesiometer, the vibration sensory analyser [VSA]) was substituted for the main experiment.

- Testing of toe position sense as a measure of proprioception was deemed adequate in terms of consistency. However, technique and interpretation of results showed moderate variation between the raters. This issue was considered resolvable with the future use of a single, more experienced, data collector.

- The temperature discrimination test produced very poor reliability using the test tube method. Sources of inconsistency included the temperatures of tap water, the length of time applied to the foot, the application pressure, and the differing surface areas due to
different foot morphology and locations of skin contact. This test was abandoned and an alternative validated test (Tip therm) was used in the main experiment.

- Results for students using Romberg’s test of balance were highly unreliable. This test was therefore abandoned. No replacement was substituted as proprioceptive function could be adequately assessed by the position sense test: Romberg’s test did not add sufficiently more than was being provided by the position sense test to warrant its continued use.

### 4.4.4 Wound assessment

The simple yet comprehensive Saint Elian scoring system permitted a very high level of repeatability after adequate education about its use, and was therefore regarded as valuable to carry forward to the main experiment.

### 4.5 Limitations of the study

Limitations of this study are its small numbers, hence rudimentary statistics, and the difficulties inherent in managing multiple data collectors, and in particular student data collectors.

### 4.6 Chapter summary

Some preliminary conclusions could be inferred from this pilot study both about the suitability of these tests for the larger trial and about the reliability and validity of these measures when in used in a student clinic setting. Most of the test elements proved to be reliable and valid in the context of this pilot study, and therefore were regarded as suitable to carry forward to the main experiment. The methods that emerged as problematic were modified or abandoned.

The issues of this pilot study provided significant experiential learning for the researcher and were used to improve the quality of the design of the main experiment. One of the outcomes was a decision to have a single data collector for the main study in order to obtain the greatest consistency in results.
The new technology involving automated portable PPG toe blood pressure testing produced reliable results in contrast to some other methods accepted in podiatry teaching and practice. There are implications for these findings in both teaching and clinical applications. Further work in this area, set up for more rigorous statistical analyses, with more systematically programmed educational design, and with larger sample numbers is recommended.
5.1 Introduction

This study was designed in light of the ulcer healing cases described in Chapter 2, the potential benefits of GTN evident in the literature review presented in Chapter 3, and the findings of the pilot study conducted in Chapter 4. Its aim was to determine the effectiveness of transdermal GTN patches for increasing TBI when pedal ischaemia is present.

5.1.1 The proposed inquiry

The study was planned to span 6 months with seven visits for each participant and the time between visits intended to be 4 weeks. The duration of the intervention was 5 months, spanning Visits 2 to 7. It was a partially randomised, participant blinded controlled experiment with 100 participants, all with peripheral artery disease (PAD), divided into four groups consisting of two intervention and two nonintervention groups. The intervention groups received either a 1.25 mg. or a 2.5 mg dose of transdermal GTN. The nonintervention groups comprised a placebo patch group and a control group with no treatment. It was originally intended that this enquiry be a randomised controlled trial (Level II evidence according to NHMRC criteria). However, because the randomisation process was compromised (details are provided below) it was downgraded to the less rigorous “experiment” status. This reduced the level of evidence that could be delivered by the study from Level II to Level III–2 as a partially randomised experiment.

This study was designed primarily to explore both short and longer term effectiveness of GTN on TBIs and to capture any effects of variation in climatic conditions. The data collection period spanned 19 months and therefore significant seasonal variation, including two winters. The location of the study in inland Australia offered climatic variations with summer temperatures at times reaching maximums in the mid 40s, and daily winter minimums down to single figures. Although most people live in environments where the
temperature extremes are controlled, the study was designed to monitor effects on pedal vascular perfusion related to these seasonal variations.

The primary outcome variable for this trial was the toe brachial index (TBI). Although this measure is recommended in international guidelines (see Appendix B), there is little procedural consensus in the literature for measuring TBIs. Furthermore, little information is available about their characteristics, and normal and normative values are still being established. Therefore it was necessary to explore the nature of TBIs in some depth to determine the most valid approach to analysis. In doing so, a number of characteristics concerning these were revealed. Investigation of these characteristics proved to be essential for the validity of the analyses and interpretations reported in this chapter.

One foot only was designated for treatment, and in all but two cases this was the foot with the lower blood pressure. In addition, the single treatment side was chosen to mirror the most common clinical scenario of vascular problems being concentrated on unilateral presentations, and the complexities of effective dose determination were simpler to navigate with a single treatment side. The non-treatment side would act as a form of individual control and help to answer the question of local versus systemic action of the peripheral application of GTN.

Additional outcome variables to TBIs were neurological tests concerning pain, protective sense, vibration sense, position sense, and temperature discrimination; and wound healing.

Blood tests for members of the two intervention groups were sought for several reasons. A full blood count was included as a check on general health to screen for any relevant underlying pathology, such as reduction in haemoglobin that would impair GTN elimination and effective nutrition and oxygenation of the blood affecting healing potential.

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1 This was done in order to determine if it was possible to demonstrate a treatment effect on the subject’s lowest TP side relative to the contralateral side. In the two exceptional cases, the higher TBI side was treated because the participants had symptoms only on the foot with the higher toe pressure (one had a motor neuropathy involving foot drop, and the other had neurological pain – painful diabetic neuropathy) and it was important for study participation with these subjects that this was the side chosen for treatment to address their desire to explore the potential for symptom relief.
A liver function test was included primarily to determine the ability to metabolise the drug, and kidney function was checked for the ability to eliminate it. A plasma GTN level assay was included with the aim of acting as a check on absorption to determine information about therapeutic and subclinical effect levels.

5.1.2 The intervention: GTN dose determination

Before commencing this research it was necessary to consider what GTN dosage rates would be effective and safe to trial. The issue of impaired absorption associated with reduced vascular supply of the periphery added complexity to the choice of the most appropriate minimum GTN dose to use in the experiment. There were some related precedents in the literature of controlled doses of GTN used in the periphery for Raynaud’s phenomenon and for painful diabetic neuropathy, but the effective dose range had not been determined for people with PAD.

The two doses chosen to use in the experiment were 1.25 mg, (the lowest dose that had been found to be of value for use on the extremities) and 2.5 mg (the maximum dose that was reported to be under the threshold for tolerance). The next two paragraphs detail the literature that contributed to the choice of each of these doses.

The work of Paoloni and his colleagues (Paoloni, Appleyard, Nelson, & Murrell, 2005; Paoloni & Murrell, 2007; Paoloni, Murrell, Burch, & Ang, 2009) contributed to the determination of doses used in the study. With regard to lower GTN thresholds, they found that in cases of chronic noninsertional Achilles tendinopathy, a dose of 1.25 mg was effective (Paoloni et al., 2005). The continuous use of low doses of 1.25 mg and 0.74 mg were also effective for epicondylitis (tennis elbow), but two higher doses (1.44 and 3.6 mg) were not (Paoloni & Murrell, 2007). In their 3-year follow up of GTN on Achilles insertional tendinopathy, a wide range of doses and application regimens was used, from 1.25 mg/24 hours to 5 mg/24 hours. Applications varied from 8 hours a day to continuous patch application. The researchers concluded that all GTN users experienced reductions in pain symptoms despite varying doses and regimens (Paoloni et al., 2009). A summary of the literature regarding continuous treatment indicates that higher doses do not provide better results. Higher doses quickly exceed the tolerance threshold for
continuous treatment, then lose all effect, as demonstrated by both Wei et al. (2011) and Paoloni and Murrell (2007).

In establishing the maximum dose for this study, the issue of tolerance predominated. The problem of tolerance with GTN means that doses above 2.5 mg require a break in their use after about 14 hours, with a rest period of about 10 hours to reinstate drug effect. This is because of depletion of thiols in the biochemical pathways of action. To find a therapeutic dose for wound healing in ischaemic feet, the dose that would provide uninterrupted and extended improvement in perfusion for an individual would be ideal, with effects maintained consistently across a full 24 hours. A dose below the reported threshold of tolerance was considered most suitable as it could be worn constantly for continuous provision of its vasodilatory and other effects. The 2.5 mg dose was reported to be the maximum dose that did not result in tolerance according to Wei et al. (2012). However, this was determined in the context of doses for angina, and therefore was tested on the chest. This dose was also used on feet of people with diabetes as recommended by Rayman, Baker, and Krishnan (2003) in their regimen for the treatment of PDN. These authors recommended titration down to 2.5 mg for each foot after a test of 5 mg on one foot for side effects. The dose of 2.5 mg was chosen as the larger dose for this study due to this evidence.

GTN is known to be generally well tolerated in terms of minimal side effects (Jorge, Feres, & Teles, 2011; Merck Sharpe & Dohme Corp., 2014). When side effects do occur, the commonest are headache, flushing, and dizziness. Side effects are dose related, but the low dosages used in this study, applied to an extremity, were expected to produce little or no systemic effect and therefore a low occurrence of side effects (see Herrick, 2009; Santoro et al., 2000; Uxa et al., 2010).

5.1.3 Research questions and hypotheses

As indicated above, this study was designed to test whether clinically measureable increases in pedal vascular perfusion, as well as neurological and wound-healing benefits, could be found in a cohort of people with pathologically low toe pressure by the use of two specific low doses of transdermal GTN. The measurement of TBIs was explored because of the
evidence recommended in guidelines (Norgren et al., 2007; Rooke et al., 2011) that TBIs were likely to be the most accurate indicators of peripheral pedal vascular sufficiency. Therefore, a number of questions—some of which arose in light of the literature cited in previous chapters, and some that seemed to be of prospective importance despite lack of prior enquiry—concerning the measurement of TBIs were posed:

- What is the number of TBI readings needed to achieve a valid measurement?
- Is the first of three TBI readings lower than the other two?
- What is the influence of cuff sizes on TBI measurements?
- What is the difference in TBI measurement between the two feet?
- What is the stability of TBIs over time?
- What are the relationships of TBIs with demographic and health variables such as age, smoking, BMI, medical conditions, and medications?
- What are the effects of climatic temperature on TBIs?

Additional questions were initially posed regarding the influence of GTN on pedal blood pressures, neurological function, and wound healing. These questions are presented under the three main hypotheses specified below.

**Hypothesis 1**

In people with subnormal TBIs, transdermal GTN at doses of 1.25 mg and 2.5 mg will cause measureable increases in TBIs.

**Associated questions**

- At 1 month will the 1.25 dose be more or less effective than the 2.5 dose in increasing TBIs?
- At 5 months, will the 1.25 dose be more or less effective than the 2.5 dose in increasing TBIs?
- What is the occurrence of dose-related side effects with GTN for 1.25 and 2.5 mg?
- Are there local and/or systemic effects of GTN?
• Will GTN be more effective in increasing toe pressures with warmer climatic conditions and less effective in colder temperatures?

**Hypothesis 2**

In people with subnormal TBIs and neurological impairment, transdermal GTN at doses of 1.25 mg and 2.5 mg will cause measureable improvements in neurological indicators of pain, protective sense, vibration sense, position sense, and temperature discrimination.

**Associated questions**

• At one month will the 1.25 dose be more or less effective than the 2.5 dose in improving neurological indicators?

• At 5 months, will the 1.25 dose be more or less effective than the 2.5 dose in improving neurological indicators?

• Will GTN be more effective on neurological indicators with warmer climatic conditions and less effective in colder temperatures?

**Hypothesis 3**

In people with subnormal TBIs, transdermal GTN at doses of 1.25 mg and 2.5 mg will cause measureable improvements in would healing.

**Associated question**

• Will GTN be more effective on wound healing in warmer conditions and less effective in colder temperatures?

5.1.4 Study location

As for the pilot study, campuses of Charles Sturt University (NSW Australia) provided the main study setting. The clinic located on these campuses contributed human and physical resources, specifically the support of academic and general staff, many of the clinic’s existing clients, and the appropriate clinical space, equipment, and facilities needed for conducting the study.
5.1.5 Ethics approval

Ethics approval was sought from the Human Research Ethics Committee (HREC) of Charles Sturt University and was granted on 8 November 2011, protocol number 2011/146. Refer to Appendix A.

5.1.6 Trial registration

The trial was registered with the Australian New Zealand Clinical Trials Registry on 20 August 2012. The trial number is ACTRN12612000883819. Web address of trial: http://www.ANZCTR.org.au/ACTRN12612000883819.aspx

5.2 Methods

One foot only was designated for treatment, and in all but two cases this was the foot with the lower blood pressure. In addition, the single treatment side was chosen to mirror the most common clinical scenario of vascular problems being concentrated on unilateral presentations, and the complexities of effective dose determination were simpler to navigate with a single treatment side. The nontreatment side would act as a form of individual control and help to answer the question of local versus systemic action of the peripheral application of GTN.

Additional outcome variables to TBIs were neurological tests concerning pain, protective sense, vibration sense, position sense, and temperature discrimination; and wound healing.

Blood tests for members of the two intervention groups were sought for several reasons. A full blood count was included as a check on general health to screen for any relevant underlying pathology, such as reduction in haemoglobin that would impair GTN elimination and effective nutrition and oxygenation of the blood affecting healing potential. A liver function test was included primarily to determine the ability to metabolise the drug, and kidney function was checked for the ability to eliminate it. A plasma GTN level assay was included with the aim of acting as a check on absorption to determine information about therapeutic and subclinical effect levels.
5.2.1 Medical practitioner involvement

At the commencement of recruitment, a generic letter was sent to all 300+ local general medical practitioners. See Appendix H. This letter had three purposes: first to act as an introduction and a courtesy to inform the local doctors of the impending study; second, to advise them that they would be sent an individualised letter relevant to the study needs of any of their patients who presented as suitable for inclusion in the study; and third, to invite them to refer any suitable patients for inclusion as potential study participants.

A second individualised GP letter, sent if their patient became a potential study subject by meeting all the inclusion criteria (see Appendix H), comprised:

- a request for the doctor’s confirmation about the suitability of their specific patient to participate,
- notification of their patient’s allocated study group,
- provision of complete inclusion/exclusion study criteria, and
- for those in the active GTN treatment groups, GTN prescribing information and a prescription request for both GTN patches and relevant blood pathology tests.

This second letter was produced in three variations: one for active treatment groups, with one of two specific doses indicated; one for placebo participants; and a final one for control group participants. The GPs were therefore not blind to the group allocation of their patients. This was essential in order for them to prescribe GTN for the active group participants and also to be fully informed so that they could act in the best interests of their patients in terms of overall medical management.

A form letter (also in Appendix H) was used for any necessary communications that occasionally occurred during the study time. A final report (also see Appendix H) was supplied at the conclusion of the experiment for each participant’s doctor. This contained the outcomes of the trial specific to that participant. This letter indicated that additional information stemming from the analysis would be sent by mail with information about publications emerging from the study. There was, in addition, another document sent to
participating GPs, which was a page included with the final patient specific letter. It was a summary of the interim findings of the study to date. This was updated as the study progressed.

5.2.2 Participants

A minimum number of 80 participants was determined as being necessary for adequate statistical power (K. Russell, personal communication, 14 July 2011). However, additional people were enrolled to provide a buffer that gave the security of extra numbers if attrition occurred from the sample. Approximately 340 people were screened to find those who fulfilled the selection criteria. Refer to Section 5.2.4.1.1 below for more details.

The calculation of sample size required standard deviation of toe pressures in the population with PAD to be studied. However, a population standard deviation could only be estimated given that this information did not yet exist for TBIs in the population being studied.

It was concluded that a sample size of 80 would give adequate power based on the formula for calculating this. It was decided that a P value of .05 was desirable to indicate significance, and that power of 80%-95% could be considered adequate. In terms of effect, a TBI change of .15 over 6 months was suggested as a minimum to indicate some meaningful change. However, a TBI change of >.2 could be considered a very meaningful effect.

To accommodate for some of the unknown elements in this investigation, recruitment was extended to 123 to give the extra power and the leeway of an extra 43 participants, allowing also for 10-12% attrition.

5.2.2.1 Sources of participants

Many of the participants were drawn from the clients already attending the CSU podiatry service at the Albury campus of the Charles Sturt University Allied Health Clinic, and others were recruited from the local community. The local diabetes support group invited

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2 At the time, Dr Russell was a statistician at Charles Sturt University.
each of its 200 members via a purpose-specific mailout. Local media were also used for recruitment in the forms of newspaper and television news coverage of the project that made requests for volunteers for the study.

5.2.2.2 Inclusion/exclusion criteria and rationales

Inclusion criteria (with rationale in italics):

- Able to speak and comprehend spoken English. *This 2 way communication ability was essential as access to a range of interpreters was not readily available.*

- Aged 18 or over. *This project looked exclusively at adults with measurable peripheral arterial disease, which is primarily a problem of mature and ageing populations although some younger adults qualified for the study.*

- A toe brachial index (TBI) of \( \leq 0.65 \) on at least one foot (\( \leq 80 \text{ mm Hg} \) was the alternative if using a small cuff). *These thresholds for inclusion indicated significant vascular impairment.*

- Willing and able to give informed consent to the elements of the study. *This element was essential to satisfy ethical guidelines and ensure complete understanding.*

- Willing and able to comply with the commitments of participation in the study, including the use of a patch on one foot for 5 months and replacement of this patch daily. Carers were able to assist with this if necessary. *Willingness and ability to adhere to the daily routine of patch wearing and daily replacement was necessary for a study participation commitment. If a carer was to be involved in the routine, their supplementary informed consent and signature was required on the consent document in addition to that of the main participant.*

  - Attendance for one assessment and six measurement visits over 6 months. (More visits were required if wounds were present. Referral to the Charles Sturt University podiatry service was planned where appropriate for supplementary wound care).

  - Willing to have a single blood test. *Participants in the active treatment groups were required to have only one blood test.*
Exclusion criteria (with rationale in italics):

- Disabling psychiatric conditions including dementia.

- Leg wounds above the ankle (foot wounds not excluded). Chronic leg wounds above the ankle are routinely associated with venous insufficiency and associated oedema, which contributes to venous stasis.

- Currently using glyceryl trinitrate in any form. This includes patches and sublingual spray as treatment for angina.

- Using Sildenafil (Viagra), Tildenafil (Cilalis), or Vardenafil (Levitra). A profound and generalised hypotension (drop in blood pressure) may occur, associated with physical collapse and loss of consciousness. This can constitute a medical emergency.

- Very high blood pressure-uncontrolled hypertension of > 160 mm Hg systolic or > 110 mm Hg diastolic pressure readings. People with brachial blood pressure in these high ranges are unsuitable for the trial due to the typically high fluctuations in BP. These people would be on a range of antihypertensive medications that may need adjustment in dose and type to best control their hypertension. This would be a source of inconsistency in toe pressure results.

- Very low blood pressure - Brachial hypotension of < 100 mm Hg systolic. These people would be at risk of dangerously low levels of hypertension, incurring the risk of falls, syncope and loss of consciousness, which may be worsened by the addition of GTN, especially if it had general rather than local effects. These low levels may also be associated with greater than usual excursions in the blood pressure generally, which could affect the consistency of toe pressure readings.

- Heart failure associated with myocardial insufficiency due to obstruction, hypertrophic cardiomyopathy, aortic or mitral stenosis, or pericarditis. These conditions are contraindications for GTN use. The symptoms of these conditions may be worsened if the overall blood pressure is lowered under a functional threshold for the optimal cardiac capacity. The reduction in overall blood pressure may cause increased cardiac stress by triggering an increase in heart stroke rate.
• Increased intracranial or intraocular pressure- glaucoma, *GTN is contraindicated in these conditions as vasodilatation could cause exacerbations.*

• Marked anaemia. *GTN use is contraindicated as documented in the prescribing information for GTN.*

• History of allergy to GTN, patch adhesives, or patch components.

• “Hot spots” on either foot that exceed 4°C relative to the rest of the foot or the opposite foot. *Relative temperature increases associated with incipient ulceration and Charcot foot. Effects of GTN unknown in these presentations.*

5.2.3 Measurements and instruments

This section contains information about the instruments used to obtain demographic data and medical history from participants, and information about the clinical tests. The clinical tests fall into the same categories that were used in the pilot study in Chapter 4, namely temperatures, vascular assessments, neurological assessments, and wound assessment. The following assessment items used the same test methodologies as described earlier in the pilot study. They are room and skin temperatures, the vascular assessment items of brachial blood pressure and TBIIs, pain, protective sensation and position sense.

New items additional to the pilot study were climatic temperatures, the electronic neurothesiometer test for vibration sense (the VSA), the Tip Therm temperature discrimination test, and blood pathology testing.

5.2.3.1 Demographic data

Personal contact details comprising the subject’s name, sex, date of birth, address, and GP contacts were collected. Demographic details that affect health were also recorded, namely whether currently smoking, smoking history, quantity of current alcohol consumption, BMI, and activity levels.

5.2.3.2 Medical history
Participants were asked whether or not they had diabetes mellitus, and, if so, the years since diagnosis as well as the latest HbA1c test results if available. The presence or history of any foot ulcers, previous toe or foot amputation, and Charcot’s disease was recorded. Heart disease was recorded in the domains of previous myocardial infarction (MCI), cerebrovascular accident (CVA), carotid artery disease (CAD), and atrial fibrillation (AF). Cardiovascular surgery and lower limb surgery were also noted. Other related medical conditions were recorded, namely rheumatoid arthritis (RA), connective tissue disease (CT), Raynaud’s disease, and a history of chilblains.

Although osteoarthritis is not a vascular related issue in terms of its primary pathology, the effects of osteoarthritis in weight-bearing joints on mobility limitation create a link to vascular disease by increasing the associated risk of reduced activity. The presence of osteoarthritis in weight-bearing joints and spine was therefore included. (Rahman, Kopec, Cibere, Goldsmith, Anis (2013)

5.2.3.3 Medications

Information about participants’ current prescribed medications was collected. The details of cardiovascular and related medications of the following drug classes were recorded:

- Antihypertensive types: ACE inhibitors, angiotensin 2 blockers, calcium channel blockers, beta blockers, diuretics, other types of antihypertensives
- Cardiovascular medications: antiarrhythmics, warfarin, aspirin, heparin, hypolipidaemics, antiangina medications
- Non steroidal anti-inflammatory drugs
- Insulin
- Oral antihyperglycaemics
- Number of any other medications.

5.2.3.4 Temperature assessments
As in the pilot study, both room and skin temperatures were recorded in this experiment, with the additions of the maximum and minimum daily (climatic) temperatures. It was anticipated that all three could be regarded as independent variables or covariates in the analyses. In addition, skin temperatures were assessed to determine whether foot warming would be necessary to obtain valid vascular measurements and as screening for higher temperature (“hot spots”) possibly representing incipient pathology.

5.2.3.4.1 Room temperature

A thermometer was placed in the clinical room to monitor room temperature where the measurements were taken.

5.2.3.4.2 Skin temperatures

To monitor for hot spots and the pathology they may represent (see Chapter 3, Section 3.6.3), this study incorporated skin temperature monitoring with the Exergen skin temperature thermometer at three sites that were chosen for their clinical usefulness.

5.2.3.4.3 Climatic temperature

The maximum and minimum climatic temperatures for each day were sourced from records of the nearest weather recording station (Albury Airport), accessed through the statistics of the Australian Bureau of Meteorology.

5.2.3.5 Vascular assessments

The vascular tests were the same as those used in the pilot study. The tibialis anterior pulse palpation and Doppler audio examination were conducted with the same grading systems of 0–3. TBIs of the hallux were obtained with the portable automated PPG (photoplethys-mography) device—the “Systoe”.

5.2.3.6 Neurological assessments

Pain score on a VAPS (visual analogue pain scale) was recorded as in the second part of the pilot study, with subjects asked to rate their most severe pain experience of the previous week.
Protective sensation was tested, as in the pilot study, with the validated Semmes Weinstein 10 g monofilament at 10 sites. The standard Carville protocol was used for monofilament testing.

Vibration testing for deep pressure and vibration sense was performed with an electronic neurothesiometer, the vibration sensory analyser (VSA [Medoc Israel]; Shy et al., 2003). The VSA has been validated to measure sensitivity to a vibratory stimulus. It involves a stimulus of increasing intensity on the great toe, where the test subject is required to indicate when the stimulus is first perceived. The measurement occurs when the foot is resting on a platform placed so that a vibrating button is located under the pulp of the great toe. The stimulus occurs five times over a 2–3 minute period. The first test result is omitted from analysis as an acclimatising run in accordance with the validated protocol. The subsequent four test results are averaged to form the subject’s score.

Position sense was assessed. In cases of significant limitation of the test joint of the great toe (the first metatarsophalangeal joint), which occurred occasionally, the more distal interphalangeal joint was used and was considered an appropriate alternative.

Temperature discrimination was assessed with the Tip Therm device (GmdH [Germany]), as described by Viswanathan et al. (2002). According to these researchers, this device has high levels of specificity and sensitivity report 100% sensitivity for this test of loss of temperature discrimination as a sign of sensory neuropathy. It is a 10 cm rod with opposing ends of metal and plastic. The difference in the perceived temperature when applied to sensate skin is due to the relative conductivity of the metal end (perceived as colder) contrasting with the relatively insulating plastic end (perceived as warmer). This temperature difference is 10 °C, which is the diagnostic threshold of clinically significant neuropathy (Viswanathan et al., 2002). Room temperature is important for the validity of the Tip Therm. In ambient temperatures greater than 23 °C, this test becomes invalid.

Alternate ends of the Tip Therm device were applied at the same three sites from where temperatures were taken. At each set of applications of each of the different ends, a forced
choice question was asked for the participant to distinguish between the colder of the two stimuli. The subjects were asked using the following words “Which feels the coldest, 1 or 2” as the plastic and metal ends of the test rod were alternated against the most distal flat patch of non plantar skin. This was repeated up to three times if results were inconclusive or conflicting from the first two trials at each site until the presence or absence of temperature discrimination was determined.

On the small number of occasions that the room temperature exceeded 23 °C, the device for temperature discrimination was cooled by briefly placing it in a cup kept refrigerated for the purpose, along with a thermometer until the temperature of the device was 2–3° lower than 23 °C.

5.2.3.7 Wound assessment

Wound classification was performed using the Saint Elian Wound Score System (Martinez-De Jesus, 2010). Measured photography was included in wound monitoring.

5.2.3.8 Blood pathology testing

Four blood tests were included for subjects in the active treatment groups. It was considered justifiable to request these tests for only the GTN intervention groups. These were a full blood count, liver function, kidney function, and a GTN plasma assay. Rationales for these are provided in Table 5.1. Participants were scored as normal or abnormal in each of the first three domains.

Table 5.1

<table>
<thead>
<tr>
<th>Test</th>
<th>Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full blood count</td>
<td>Screening for haematological abnormalities that could affect test results such as iron levels</td>
</tr>
<tr>
<td>Liver function</td>
<td>Important for drug metabolism</td>
</tr>
<tr>
<td>Kidney function</td>
<td>Important for drug excretion and elimination</td>
</tr>
<tr>
<td>GTN plasma assay</td>
<td>Included to give dose related information about absorption and effectiveness of the GTN intervention</td>
</tr>
</tbody>
</table>
The assay of GTN in blood plasma was to be conducted with blood from the same single blood collection episode. This test was not available though any commercial pathology laboratory. However, assistance from Charles Sturt University scientist Dr Hassan Obied was obtained with the purpose of performing this test.

5.2.4 Procedure

The data were collected solely by the primary researcher with the exception of eight episodes in which students were involved who had earlier taken part in the pilot study. These students were supervised by the primary researcher during these eight joint data collection episodes which constituted less than 1% of the total data collected.

For all visits, temperatures of the test environment were maintained as close as possible to 22 °C and within the recommended range of 21–25 °C for vascular measurements as described in Chapter 4. These temperatures were recorded from thermometers in the assessment rooms. The study was conducted at two different sites as the university moved the location of its clinical facilities within the period of the data collection. It was possible with air-conditioning and heating to maintain the temperature at close to 22 °C in the clinic at both facilities. An exception occurred during the summer of the first year of data collection, when temperatures sometimes rose to 25 °C in the test environment.

A commercially available foot warmer was used according to the recommendations in the Cloete (2009) study, for 5 minutes or longer if needed when toe pressures were unprocurable to raise the skin temperature to at least 20 °C. This method increased toe pressures to measurable levels. There was a very small number of occasions on which warming was performed due to unprocurable TBIs in the coldest winter temperatures \( (n = 3) \) and these occasions were specifically flagged, but this small number was not useful for any further analysis.

The study was planned to span 6 months with seven visits for each participant and the time between visits intended to be 4 weeks. The duration of the intervention was 5

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3 Dr Obied (BPharm, PhD, MRACI, CChem) is a senior lecturer in pharmacology at the university.
months, spanning Visits 2 to 7. It was a partially randomised, participant blinded controlled trial with 100 participants, all with peripheral artery disease (PAD), divided into four groups consisting of two intervention and two nonintervention groups. The intervention groups received either a 1.25 mg. or a 2.5 mg dose of transdermal GTN. The nonintervention groups comprised a placebo patch group and a control group with no treatment. It was originally intended that this enquiry be a randomised controlled trial (Level II evidence according to NHMRC criteria). However, because the randomisation process was compromised (details are provided below) it was downgraded to the less rigorous “non randomized experimental trial with evidence level classification level III-2”. This reduced the level of evidence that could be delivered due to its partial randomisation.

This study was designed to explore both short and longer term effectiveness of GTN on TBIs and to capture any effects of variation in climatic conditions. The data collection period spanned 19 months and therefore significant seasonal variation, including two winters. The location of the study in inland Australia offered climatic variations with summer temperatures at times reaching maximums in the mid 40s, and daily winter minimums down to single figures. Although most people live in environments where the temperature extremes are controlled, the study was designed to monitor effects on pedal vascular perfusion related to these seasonal variations.

5.2.4.1 Procedures at Visit 1 (baseline)

5.2.4.1.1 Selection of participants

People were assessed in an initial visit during which their suitability as participants was determined according to the inclusion and exclusion criteria. As mentioned above, approximately 340 people were screened for prospective inclusion, and 122 were selected for participation. Of those excluded, by far the most common reason for ineligibility was both TBIs being over the inclusion threshold of PAD (i.e., both TBIs > 0.65). The next most common reason for ineligibility was brachial blood pressure > 160 mm Hg. All people with potentially pathological BP measurements were referred to their current general
medical practitioners for review. Some of these returned for revision and were subsequently included in the study after medication adjustment had controlled their blood pressure.

5.2.4.1.2 Allocation of participants to groups

The 122 people who were regarded as eligible were allocated to one of the four groups by a partly randomised process. This was achieved by having the numbers 1–4 (printed in yellow ink to ensure their complete concealment in opaque paper envelopes) being randomly placed in sealed envelopes prior to the person’s group allocation, with a study number written on the outside of each envelope. During the assessment process, as each person fulfilled the inclusion criteria the next consecutively numbered envelope was allocated to them, and the number 1–4 within was revealed.

This process determined both the participants’ study number and their group allocation. It was sometimes witnessed by assisting clinical podiatrists at the university clinic. However, there were a number of occasions on which I overrode this allocation process and placed people who were symptomatic or at highest risk into an active treatment group. The motivation for this was a combination of clinical ethics and personal morals. I found I was not always able to withhold GTN treatment while believing it to be potentially useful when people were symptomatic or at high risk. This had several consequences. It led to greater numbers of participants in the active treatment groups as well as a preponderance of healthier participants with less clinical need in the placebo and control groups. It also might have influenced the research outcomes in that it could have led to Type II errors in failing to find effectiveness of GTN when one indeed existed. However, it also was creating a situation in which, if a treatment effect was determined in the groups with more pathology relative to the controls, there would be an even stronger indication that GTN fulfilled its potential in improving vascular supply for people with PAD and related problems.

All participants were provided with information about the research and were asked to sign a consent form. See Appendices I and J. People allocated to an active patch group were directed to see their GP, taking a letter requesting their prescription for GTN and blood pathology services (see Appendix H). Placebo group participants were instructed to go to the participating pharmacy with a sealed letter containing my request for dispensation.
of their placebo patches (also see Appendix H). This had been prearranged with the pharmacy, and it was informed via email in advance of the presentation of the participant.

5.2.4.1.3 Assessments conducted

As part of the selection processes for inclusion in the study, baseline vascular data, including TBIs, were obtained at the first visit. As in the pilot study, vascular assessments were taken after a 10-minute recumbent rest period. If any wounds were present, treatment was commenced immediately.

5.2.4.2 Procedures at Visit 2

5.2.4.2.1 Assessments conducted

The second visit for all eligible participants, 4 to 6 weeks later, involved the collection of baseline demographic information; medical history data; neurological data comprising the pain, protective sense, vibration sense, position sense, and temperature discrimination; and wound assessment data. At this second visit vascular measures were not taken from members of the intervention or placebo groups because time was taken to explain their use of patches (see below). However, in the control group where the lack of necessity to commence patches allowed for more time, vascular measures were sometimes performed.

5.2.4.2.2 Instructions for participants about use of patches

The second visit was also used for the commencement of patch application in the two intervention groups and the placebo group. A document was given to all of these subjects with information about use of the patches. This was personalised with their name and which foot to use. See Appendix I. The foot with the lower baseline toe pressure was identified to the participant as the one for patch application.4

In order to obtain the correct dose, the intervention group subjects were instructed how to cut patches into halves or quarters depending on their group allocation. (The dose is directly related to the surface area of the patch and the two fractional doses were obtained by cutting the 5 mg patches into segments.) The low GTN group was instructed to use a

4 See Footnote 1 in this chapter (Section 5.2.1) with regard to two exceptions.
quarter of a circular 5 mg patch (1.25 mg). The high GTN group was instructed to use half of a 5 mg patch (2.5 mg).

The placebo group was provided with patches made using the same adhesive as the GTN patches. It was provided by a local sticker manufacturing company using photographic technology on a paper sticker. Vinyl stickers were considered for the trial, which would have made an even better duplicate of the plastic GTN patch. However, the chemical components in vinyl were found to be unsuitable for prolonged skin contact due to possible health risks from some components. The placebo patches were all pre-cut into quarter circles but otherwise were visually very similar to the medicated patches.

Advice about how to use the patches was given to participants in all three groups, and observations were made of them applying their first patch after instruction. They were asked to apply a fresh patch each day to the dorsum of their treatment foot only (see photographs with patches on feet in Chapter 2, Figures 2.18, 2.21, and 2.23) and they were advised to rotate the patch daily to a new area of skin to minimise any chance of skin sensitivity reactions.

Subjects in the intervention groups were made aware of the possibility of side effects, with the most likely being headaches. It was explained that headaches should be responsive to simple analgesics and that a reversal of any effects should occur on removing the patch, with complete cessation of any effect by 2 hours after removal. They were advised that any effect after this 2-hour period would be unlikely to be due to the patch and that they should seek medical advice for any ongoing concerns.

5.2.4.3 Procedures at Visits 3 to 7

Visits 3 to 7 were routine repeat monitoring visits where vascular and most neurological measures were obtained at each visit. Apart from the initial baseline assessment taken at Visit 2, neurothesiometer (VSA) measures for vibration sense were taken only at Visits 4 and 6.
Side effects were monitored with questioning regarding headaches and observation for skin changes at each visit. If any wounds were present, they were documented and cared for using best practice guidelines.

5.2.4.4 Analyses

The foot with the lower TBI at baseline was referred to as the LbTBI side for the purposes of data identification and analysis. This foot was the side treated with the GTN intervention in the active intervention groups. The foot with higher TBI at baseline was referred to as the HbTBI side. It was the nontreated side for those in the intervention groups. There were, however, two exceptions to this rule where for reasons specific to the needs of the participants, the higher-pressure foot received the intervention.

Preparation and analysis of the data involved a variety of procedures. Data were initially entered into Excel spreadsheets. They were subsequently reconfigured and anonymised, then transferred and analysed within SPSS Version 20 (IBM, 2011). Raw data, boxplots, scatterplots, and histograms were inspected visually. Descriptive statistics were used to obtain aggregated data, percentages, percentiles, minimum and maximum values, absolute score values, indices of skewness and kurtosis, difference scores, means, standard deviations, correlation coefficients, intraclass correlation coefficients, percentages of variance accounted for, effect sizes, and confidence intervals.

Both parametric and nonparametric inferential statistics were used, depending on whether the data brought into the analyses were equal interval or categorical; the data were normally or non-normally distributed; or whether there were (approximately) equal, or noticeably unequal, numbers in the groups being compared. Inferential statistical tests used were the Spearman’s rank-order correlation coefficient, chi-squared analysis, Fisher’s exact test, Mann-Whitney U test, Wilcoxon matched-pairs signed-ranks test, Kolmogorov-Smirnov and Shapiro-Wilk tests of normality, the student’s t-test, Levene’s test of variance, ANOVA, and ANCOVA. Results were regarded as being statistically significant when two-tailed p values were less than .05. Participant records were retrieved in some instances to enhance interpretation of results.
The data collected at Visit 1 (baseline), Visit 3 (1 month after commencement of the intervention), and Visit 7 (5 months after commencement of the intervention) were taken forward for further analysis. The baseline–Visit 3 timespan was chosen to provide information about short-term effects of the intervention, and the baseline–Visit 7 timespan was chosen to provide information about long-term effects.

In a small number of instances, analyses based on nonindependence of observations were used in parallel with analyses involving independence of observations to determine if these two approaches yielded any important differences (see Menz, 2004). All analyses were conducted on a per protocol basis, not according to intention to treat (ITT) because issues concerning compliance were not a focus of this study (see Armijo-Olivo, Warren, & Magee, 2009; Feinman, 2009).

5.2.5 Variables not included in analyses

Although data were collected for all of the variables detailed above, inspection of the data—both as they were being collected and upon completion of collection—indicated that some of them did not warrant bringing forward for further analysis. Details concerning the variables not included in analysis are provided in the next five subsections.

5.2.5.1 Temperatures

After the data had been collected it was realised that, although a number of participants reported cold feet during winter and were symptomatic only in winter, most participants resided in artificially heated environments during periods of seasonal cold, and those environments largely protected them from low climatic temperatures. Therefore, a relationship between climatic temperatures and other elements was not able to be demonstrated in this experiment.

The temperatures of the test environment were able to be successfully maintained as intended within the small ideal range recommended in the literature. Therefore, there was insufficient variability on this variable to warrant further analysis.
5.2.5.2 Vascular measures

Vascular test results of pulses and Doppler sounds were visually scanned and showed very little change across time or variability. Because both of these variables were scored on a four-point scale from only 0–3, measurement of them was too blunt to reflect the modest changes that might have been attributable to the GTN intervention. The most common scores were 2 for both an attenuated pulse and a biphasic Doppler sound. People qualifying for inclusion in this study with low toe pressures would not ever be likely to attain triphasic scores that are almost invariably found only in young healthy adults. While a score of 2 is widely considered a normal finding in older adults, it does not preclude the presence of vascular pathology due to its concomitant finding with low toe pressures. Monophasic Doppler sound findings and absent pulses are each significantly associated with pathology, but biphasic Doppler sounds and grade 2 pulses are both ambiguous. No further analyses were therefore pursued in with these variables.

5.2.5.3 Neurological measures

It can be difficult to differentially diagnose neurological pain, although typical patterns exist. Clearly defined neurological pain was present in the feet of only nine participants. Although there were decreases in pain for some of these participants, in essence there were not enough participants with neurological pain to warrant further analysis. Pain from all other sources was most commonly musculoskeletal pain and pain of problem nails and hyperkeratotic lesions. Overall, there were general reductions in pain associated with treatment, as patients were referred to podiatry care for any symptomatic foot pain problems. This obscured any outcomes associated with GTN intervention in regard to pain, so this was not worth further analysis in the context of this study.

The loss of protective sensation (LOPS) is a phenomenon that is linked to HBA1c and long-term diabetes control. Normal scores of 10 were very stable as were very poor scores of 0. Although some people improved in their LOPS score, the number of people with LOPS was too low (n = 20) to make analysis of the small amount of variation meaningful in this enquiry.
For vibration, the results were particularly variable, which was surprising given the nature of the validated electronic system used. As the data with the VSA were collected, it was obvious that they were highly variable and apparently unreliable. Some preliminary analysis of these data indicated that further analysis was not worth pursuing due to the wide variations.

Position sense test results did not move between the two test outcomes of present versus absent. This was very evident from a visual scan of the data and therefore it was not worth pursuing in terms of GTN effect. Only a few participants had a negative result which was associated with serious disease from which no neurological recovery was likely. Although this was included as a respected test, for this particular context it was proven to be not valuable after all.

Temperature discrimination was another test with only two categories of response, in this case either present or absent. This test reportedly has high sensitivity of 100% and is a strong sign of neurological disease from which recovery is unlikely. In this case, a lack of movement of participants’ scores across the 5-month period could be readily seen in a visual scan of the data, and therefore this variable received no further analysis.

5.2.5.4 Wound healing

Wounds were classified as any break in the skin. The wounds that occurred during the trial were minor and not chronic ulcers, consisting rather of superficial broken skin from macro or micro traumatic origins. Only three people with preexisting wounds at baseline were recruited into the study. Wounds were always treated as a priority and dealt with on the day of presentation. Referral was made for follow-up at the university clinic if necessary.

One of the three people with wounds on presentation had the low dose treatment on the wound side, and another had the high dose on the contralateral side. These two people’s wounds did not heal throughout the seven visits, although the wound scores dropped slightly in each case. The third participant was in the placebo group and had seven wounds at baseline, with three on one side and four on the other. When the wounds were offloaded and appropriately dressed, healing progressed rapidly. All seven wounds had healed by the second visit.
Seven additional people developed minor new wounds during the trial. Of these, three were in the low dose intervention group, two were in the high dose group, one was in the placebo group, and one in the control group. Of the two people in the high dose group, one developed wounds on both feet from trauma. Three of the five in the intervention groups developed wounds on the side where active treatment was occurring, and two of the five developed wounds on the side opposite to the active treatment. All of the wounds that occurred during the study healed quickly and were almost invariably healed in time for the follow-up visit 1 month later.

The combination of a small number of wounds, the invariably rapid healing of the wounds that arose during the study, and the nonhealing of two of the wounds that preexisted commencement of the study presented a complexity that was not analysable in this context. This small amount of data did not provide a clear pattern as to any definitive effect of GTN with data being divided across all groups and split across treatment versus nontreatment sides.

5.2.5.5 GTN plasma assays

Detection of GTN metabolites in plasma was attempted with assays using gas chromatographic mass spectrometer analysis. The evidence for GTN metabolites in the first few samples tested was found to be zero or only negligible traces were detectable. The technology needed to be upgraded from nano level (billionth—$10^{-9}$) of analysis to pico level (trillionth—$10^{-12}$). The technology available at the university laboratory was not adequate for this latter requirement.

5.2.5.6 Concluding remarks

Because neither neurological pathology nor the presence of wounds was included in the recruitment inclusion criteria, this resulted in inadequate numbers of subjects with these conditions. This is acknowledged as a risk that was taken with the necessary choice of a single focus of the recruitment process on a single measurable parameter, which was low toe pressure. The potential benefits of being able to make conclusions about effects of GTN on neurological pain, LOPS, and wound healing were desirable secondary outcomes that
were envisaged at the start of the project, which unfortunately had to be abandoned later when inadequate data were available. It was therefore not possible to investigate the second and third hypotheses at all.”

Because of the valid omission of temperature, neurological, and wound variables from prospective subsequent analyses, exploration of TBIs became the first of the two main foci of this enquiry. The other focus is the effect of GTN on TBIs. The focus on these two topics is reflected in the results and discussion sections that follow.
5.3 Results

This section commences, in Section 5.3.1, with information about the participants, including their flow through the experiment, details about their demographic and health status variables, and information about those who were not retained in the final analyses. Following that, Sections 5.3.2 to 5.3.7 inclusive make up a large proportion of the overall results section because TBIs are the primary dependent variable but insufficient prior information was available concerning them. The first three of these subsections (Sections 5.3.2 to 5.3.4 inclusive) focus on the nature and validity of TBI measurements. The following subsection, 5.3.5, deals with associations of demographic variables with TBIs, and Section 5.3.6 is concerned with the associations between health status and TBIs. Section 5.3.7 addresses the issue of systemic side effects of GTN relating this to the TBIs of bilateral foot responses to determine the presence of local versus systemic effects. The final two subsections (Sections 5.3.8 and 5.3.9) address the effect of GTN on TBIs.

5.3.1 Participants

As reported in Section 5.2.2, approximately 340 people were screened for possible inclusion, and 122 were recruited as commencing participants. Each of these 122 participants was assigned to one of four study groups. A diagram summarising the progress of participants through the study is provided in Figure 5.1. In this diagram, the approximate numbers of people who were initially screened and excluded are shown, as are the numbers allocated to each of the four groups. The processes and assessments for each of the seven visits are briefly outlined, and finally those lost to follow-up or excluded from the analyses because of insufficient exposure to the intervention are indicated for each group.

Throughout Section 5.3.1.1, descriptions are presented of the participants retained in the analyses. Section 5.3.1.2 contains information about those who were lost to follow-up or excluded.
Visit 1: People screened for prospective participation \((N \approx 340)\).

Recruited into study \((N = 122)\). Vascular readings (brachial BP, pulse, Doppler examination, and 3 TBIs) taken.

Excluded because failed to meet inclusion criteria or because of exclusion criteria. \((N \approx 220)\).

Assigned to low GTN group \((n = 41)\).

Assigned to high GTN group \((n = 31)\).

Assigned to placebo group \((n = 25)\).

Assigned to control group \((n = 25)\).

Visit 2: Demographic and health status information obtained. Neurological baseline measurements taken. Instructions given for application of patches as appropriate to each group.

Visit 3: Neurovascular and temperature monitoring. Screening for side effects.

Visits 4 to 6: Neurovascular and temperature monitoring. Screening for side effects.

Visit 7: Neurovascular and temperature monitoring. Screening for side effects.

Analysed \((n = 33)\). Lost to follow-up or insufficiently exposed to intervention, \(n = 8\)—see Section 5.3.1.2.

Analysed \((n = 24)\). Lost to follow-up, \(n = 7\)—see Section 5.3.1.2.

Analysed \((n = 22)\). Lost to follow-up, \(n = 3\)—see Section 5.3.1.2.

Analysed \((n = 21)\). Lost to follow-up, \(n = 4\)—see Section 5.3.1.2.

*Figure 5.1* Diagram showing flow of participants through the experiment.
5.3.1.1 **Participants included in analyses: Characteristics at baseline**

Demographic characteristics of the participants, including their health-related behaviours, prevalence of medical conditions, and medications are presented in Tables 5.2 to 5.9. This information is provided to give an overview of the participants’ characteristics and for determining the extent to which the groups were similar to each other despite the process of group allocation having been only partially random.

The first four tables contain information about the total sample as well as the two GTN intervention groups combined and two control groups combined. The subsequent four tables contain a breakdown of the same information into each of the four (two GTN intervention and two control) subgroups. A broad description of the total sample, as well as subgroups, is provided within the text. All statistically significant differences between the groups in this section (5.3.1.1) are reported within the text and summarised in Section 5.3.1.1.3 where they are condensed in Table 5.10. ANOVAs, *t*-tests, chi-square analyses, and Fisher's exact tests\(^5\) were used to determine whether differences between the groups were statistically significant or not. No adjustments were made to protect against Type I errors because use of adjustments could have resulted in a false impression of similarity across the groups. The comparisons that marginally failed to attain statistical significance, with *p* values between .05 and .08, are also reported—again within the text—to avoid creating any misleading impressions of similarity across the groups. These results are also summarised in Section 5.3.1.1.3 and condensed in a table, Table 5.11.

### 5.3.1.1.1 Total sample, GTN groups combined, and control groups combined

Information in Table 5.2 indicates that the total sample retained for analysis comprised 100 participants with a mean age of 60.8 years (*SD = 9.5*) and medical conditions typical of a sample from this age group who have known PAD. The balance of sex of participants was slightly biased toward males at 58%.

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\(^5\) Fisher's exact tests were used instead of chi square if any expected cell numbers were less than five.
Table 5.2

Demographic Characteristics of Total Sample, Treatment Groups Combined, and Placebo and Control Groups Combined (N = 100)\(^a\)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total sample ((N = 100))</th>
<th>Both intervention groups: high and low GTN doses ((n = 57))</th>
<th>Both control groups: placebo and control ((n = 43))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>69.8 (9.5)</td>
<td>70.4 (8.4)</td>
<td>69.6 (10.8)</td>
</tr>
<tr>
<td>Minimum–maximum</td>
<td>38–91</td>
<td>42–86</td>
<td>38–91</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>58 (58)</td>
<td>33 (57.9)</td>
<td>26 (60.5)</td>
</tr>
<tr>
<td>Female</td>
<td>42 (42)</td>
<td>24 (42.1)</td>
<td>17 (39.5)</td>
</tr>
<tr>
<td>Smoking current</td>
<td>6 (6)</td>
<td>4 (7.0)</td>
<td>2 (4.7)</td>
</tr>
<tr>
<td>Ever-smoked(^b)</td>
<td>44 (44)</td>
<td>27 (47.3)</td>
<td>17 (39.5)</td>
</tr>
<tr>
<td>Smoking history years(^b)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>34 (17.3)</td>
<td>37.5 (17.0)</td>
<td>28.4 (16.5)</td>
</tr>
<tr>
<td>Minimum–maximum</td>
<td>1–60</td>
<td>4–60</td>
<td>1–53</td>
</tr>
<tr>
<td>High alcohol intake(^c)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>35.1 (–)</td>
<td>31.8 (–)</td>
<td>39.7 (–)</td>
</tr>
<tr>
<td>Minimum–maximum</td>
<td>16–56</td>
<td>16–49</td>
<td>28–56</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>17.3 (–)</td>
<td>24.5 (–)</td>
<td>10.0 (–)</td>
</tr>
<tr>
<td>Minimum–maximum</td>
<td>10–35</td>
<td>14–35</td>
<td>10–10</td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>29.5 (5.9)</td>
<td>30.3 (6.7)</td>
<td>28.5 (4.7)</td>
</tr>
<tr>
<td>Minimum–maximum</td>
<td>19–50</td>
<td>21–50</td>
<td>19–41</td>
</tr>
<tr>
<td>Mobility(^d)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>17 (17)</td>
<td>12 (21.1)</td>
<td>5 (11.6)</td>
</tr>
<tr>
<td>High/normal</td>
<td>83 (83)</td>
<td>45 (78.9)</td>
<td>38 (88.4)</td>
</tr>
</tbody>
</table>

\(^a\) Values are numbers (percentages) unless indicated otherwise. A dash is placed within the parentheses where numbers are too small for standard deviations to be meaningful.

\(^b\) Based on those who have smoked in the past or are currently smoking.

\(^c\) Refers only to men consuming more than 14 standard drinks of alcohol a week \((n = 7)\) and women consuming more than seven standard drinks of alcohol a week \((n = 4)\).

\(^d\) Mobility coded 1. Low: needing assistance with activities of daily living; 2. Medium: impaired but independent with activities of daily living; 3. High/normal: able to engage in discretionary exercise activities.
Just under half of the sample (44%) had smoked at some time, which is in line with national statistics for smoking in Australia (Australian Bureau of Statistics, 2015), but only 6% were currently smoking. This is in contrast to the current rate of smoking in Australia of 18.4% of the population (Australian Bureau of Statistics, 2015). As shown in Table 5.2, there were higher numbers in each of the three smoking-related domains of the intervention groups compared with control groups. The distribution of people who had ever smoked across the groups was uneven at 47.3% and 39.5% in the combined intervention and control groups respectively (representing a 7.6% difference). Smoking years was also uneven in the same direction, with 37.5 and 28.4 years respectively (which is a 9.1 year difference). None of these differences were statistically significant, however.

Eleven per cent of participants had a weekly alcohol intake above the recommended healthy levels (14 standard drinks a week for men and 7 standard drinks a week for women⁶), which was balanced between the intervention and control groups. A small number of participants in both groups consumed high intakes of alcohol, with maximums of 49 drinks per week in the intervention groups and 56 in the control groups.

At 29.5, the mean BMI of participants was considerably higher than the healthy BMI range of 18.5 to 24.9 (State Government of Victoria, 2014) and higher than the Australian average of 27.6 (Australian Bureau of Statistics, 2012). BMIs were quite evenly matched between the intervention and control groups. Mobility in both of these broad groups was also quite well matched, being only 10% higher in the combined control groups.

More than half of the sample (58%) had diabetes, as indicated in Table 5.3. However, only a small number (5) had foot wounds, and there were very few (3) who had minor amputations prior to the trial. No participants had previously undergone a lower leg amputation (see Table 5.4).

The contents of Table 5.3 indicate that there were similar percentages of participants with diabetes in intervention and control groups (63.2% and 51.2% respectively—a difference that was not significant). The diabetes control values of 6.4 and 6.7, as measured by glycosylated hemoglobin (HbA1c), indicate that both major groups were well matched and, additionally, that the management of BGLs reflect ideal levels of less than 7%, with the overall mean being 6.5%. All participants had at least one vascular-related condition by definition given that a low TBI is an indication of vascular pathology and because a TBI of \( \leq 0.65 \) on at least one foot was an inclusion criterion for this investigation. (A more complete breakdown of the participants’ vascular conditions is provided in Table 5.4.)

A high percentage of participants (87%) had additional medical conditions, as expected, and these were unevenly distributed across the intervention and control groups at 94.7% and 76.7% respectively. This difference was significant, \( \chi^2(1) = 7.02, p = .008 \) and foreshadows potentially important inequity in the general health status between groups.

Blood testing for pathology was confined to the intervention groups. The results of these tests, shown in Table 5.3, demonstrate typical associations and distribution in a cohort combining health conditions of known influence such as diabetes and cardiovascular conditions as well as demographic variables such as elevated BMI and the 69-year mean age group of participants. The majority (64.9%) of participants had normal liver and full blood count results. Kidney pathology was detected in 12% of those who provided test results. This is predictable in such a sample where people with diabetes are predominant (58%) with a mean disease duration of 16.7 years. The indication that most participants had normal blood pathology test results, despite a mean of 16.7 years of diabetes in over half the sample, combined with the good HbA1c results with a mean of 6.5, suggests that as a whole they were well-controlled medically and were therefore generally healthier relative to people in the general population with these same health conditions (Speight, 2013).
Table 5.3
Health Status of Total Sample, Treatment Groups Combined, and Placebo and Control Groups Combined (N = 100)\(^a\)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total sample (N = 100)</th>
<th>Both intervention groups: high and low GTN doses (n = 57)</th>
<th>Both control groups: placebo and control (n = 43)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic</td>
<td>58 (58)</td>
<td>36 (63.2)</td>
<td>22 (51.2)</td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 1</td>
<td>9 (9)</td>
<td>7 (12.3)</td>
<td>2 (4.7)</td>
</tr>
<tr>
<td>Type 2</td>
<td>49 (49)</td>
<td>29 (50.9)</td>
<td>20 (46.5)</td>
</tr>
<tr>
<td>Diabetes: Years since diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>16.7 (12.9)</td>
<td>16.6 (13.6)</td>
<td>16.8 (11.9)</td>
</tr>
<tr>
<td>Diabetes control HbA1c, mean (SD)</td>
<td>6.5 (2.5)</td>
<td>6.4 (2.6)</td>
<td>6.7 (2.4)</td>
</tr>
<tr>
<td>With vascular-related condition(s)(^b)</td>
<td>100 (100)</td>
<td>57 (100)</td>
<td>43 (100)</td>
</tr>
<tr>
<td>With other medical condition(s)</td>
<td>87 (87)</td>
<td>54 (94.7)</td>
<td>33 (76.7)</td>
</tr>
<tr>
<td>Blood pathology liver(^c)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>37 (64.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal</td>
<td>6 (10.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No test</td>
<td>14 (24.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood pathology kidney(^c)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>32 (56.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal</td>
<td>12 (21.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No test</td>
<td>13 (22.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood pathology FBC(^c)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>37 (64.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal</td>
<td>8 (14.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No test</td>
<td>12 (21.1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Values are numbers (percentages) unless indicated otherwise.

\(^b\) All participants had at least one vascular-related condition, TBI ≤ 0.65 on one or both feet, because that was an inclusion criterion for the study. Most participants had additional vascular conditions. Refer to Table 5.4.

\(^c\) Only the intervention groups had blood tests, so all percentages are based on n = 57.

The entries in Table 5.4 indicate that the macrovascular diseases of myocardial infarction, atrial fibrillation, and a history of cardiovascular surgery were slightly more common in the intervention groups—and the differences were not statistically significant. Even the largest difference in percentages, which occurred with regard to
cardiovascular surgery, was not statistically significant, $\chi^2(1) = 3.05$, $p = .069$. Other macrovascular conditions, and all microvascular conditions, were evenly distributed across the intervention and control groups, and the sum of vascular diseases was balanced across both intervention and control groups.

Table 5.4

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total sample ((N = 100))</th>
<th>Both intervention groups: high and low GTN doses ((n = 57))</th>
<th>Both control groups: placebo and control ((n = 43))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of vascular-related conditions, Mean (SD)(^\text{b})</td>
<td>2.5 (1.4)</td>
<td>2.5 (1.4)</td>
<td>2.6 (1.5)</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>14 (14)</td>
<td>9 (15.8)</td>
<td>5 (11.6)</td>
</tr>
<tr>
<td>Cerebrovascular accident</td>
<td>9 (9)</td>
<td>4 (7.0)</td>
<td>5 (11.6)</td>
</tr>
<tr>
<td>Carotid artery disease</td>
<td>5 (5)</td>
<td>2 (3.5)</td>
<td>3 (7.0)</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>9 (9)</td>
<td>6 (10.5)</td>
<td>3 (7.0)</td>
</tr>
<tr>
<td>Lower limb vascular surgery</td>
<td>12 (12)</td>
<td>7 (12.3)</td>
<td>5 (11.6)</td>
</tr>
<tr>
<td>Cardiovascular surgery</td>
<td>20 (20)</td>
<td>15 (26.3)</td>
<td>5 (11.6)</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>2 (2)</td>
<td>1 (1.8)</td>
<td>1 (2.3)</td>
</tr>
<tr>
<td>Osteoarthritis</td>
<td>39 (39)</td>
<td>22 (38.6)</td>
<td>17 (39.5)</td>
</tr>
<tr>
<td>Connective tissue disease</td>
<td>2 (2)</td>
<td>1 (1.8)</td>
<td>1 (2.4)</td>
</tr>
<tr>
<td>Raynaud’s disease</td>
<td>12 (12)</td>
<td>4 (7.0)</td>
<td>8 (18.6)</td>
</tr>
<tr>
<td>Chilblains</td>
<td>13 (13)</td>
<td>5 (8.8)</td>
<td>8 (18.6)</td>
</tr>
<tr>
<td>Charcot foot</td>
<td>1 (1)</td>
<td>0 (0)</td>
<td>1 (2.3)</td>
</tr>
<tr>
<td>Foot ulcer</td>
<td>5 (5)</td>
<td>4 (7.0)</td>
<td>1 (2.3)</td>
</tr>
<tr>
<td>History of foot ulcer</td>
<td>6 (6)</td>
<td>4 (7.0)</td>
<td>2 (4.7)</td>
</tr>
<tr>
<td>Amputation</td>
<td>3 (3)</td>
<td>0 (0)</td>
<td>3 (7.0)</td>
</tr>
</tbody>
</table>

\(^a\) Values are numbers (percentages) unless indicated otherwise.

\(^b\) Entries in this top row represent low TBIs as one cardiovascular-related condition for all participants as well as any of the additional conditions listed in this table.
Table 5.5 shows that the medications most commonly taken by participants were antihypertensives followed by hypolipidaemics. The use of antihypertensive medication was 84.2% and 72.1% for intervention and control groups respectively. This 12% greater use of antihypertensive medication in the intervention groups was not statistically significant. Hypolipidaemic medication was more closely matched at 64.9% and 65.1% respectively, and again this difference was not statistically significant.

Table 5.5

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total sample (N = 100)</th>
<th>Both intervention groups: high and low GTN doses (n = 57)</th>
<th>Both control groups: placebo and control (n = 43)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antihypertensives</td>
<td>79 (79)</td>
<td>48 (84.2)</td>
<td>31 (72.1)</td>
</tr>
<tr>
<td>Hypolipidemics</td>
<td>65 (65)</td>
<td>37 (64.9)</td>
<td>28 (65.1)</td>
</tr>
<tr>
<td>Antiarrythmics</td>
<td>4 (4)</td>
<td>2 (3.5)</td>
<td>2 (4.7)</td>
</tr>
<tr>
<td>Anticoagulants\textsuperscript{b}</td>
<td>59 (59)</td>
<td>32 (56.1)</td>
<td>27 (62.8)</td>
</tr>
<tr>
<td>Antianginals</td>
<td>7 (7)</td>
<td>6 (10.5)</td>
<td>1 (2.3)</td>
</tr>
<tr>
<td>Insulin</td>
<td>26 (26)</td>
<td>15 (26.3)</td>
<td>11 (25.6)</td>
</tr>
<tr>
<td>Oral antihyperglycaemics</td>
<td>42 (42)</td>
<td>26 (45.7)</td>
<td>16 (37.2)</td>
</tr>
<tr>
<td>Number taking other medications</td>
<td>85 (85)</td>
<td>49 (86.0)</td>
<td>36 (83.7)</td>
</tr>
<tr>
<td>Number of other medications\textsuperscript{c}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>3.2 (2.3)</td>
<td>3.5 (2.4)</td>
<td>2.8 (2.2)</td>
</tr>
<tr>
<td>Minimum–maximum</td>
<td>1–11</td>
<td>1–11</td>
<td>1–9</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Values are numbers (percentages) unless indicated otherwise.  
\textsuperscript{b} Includes warfarin, platelet inhibitors, NSAIDs, and heparin.  
\textsuperscript{c} Based only on medications additional to those listed above.

Reflecting the 12% greater prevalence of diabetes in the intervention groups, 8.5% more of these participants were taking antihyperglycemics. Approximately equal percentages of participants took additional medications in the intervention groups.
(86.0%) and the control groups (83.7%). Type 1 insulin-dependent diabetes was present in only 9% of the total sample. However, an additional 17% of the sample with type 2 diabetes were also medicated with insulin, totalling 26% (see Table 5.5). The distribution of medicated diabetes was balanced across the intervention and control groups.

5.3.1.1.2 Low GTN, high GTN, placebo, and control groups

Tables 5.6 to 5.9 provide information about the spread of the variables across each of the four subgroups: low GTN, high GTN, placebo, and control.

In terms of age, means and standard deviations were similar across the four groups, with the exception that the control group had a larger standard deviation, it having both the youngest and oldest participants (see Table 5.6). Sex distributions were even in the low GTN and control groups, but were uneven with an approximate 2:1 male:female ratio in both the high GTN and control groups.

High means, standard deviations, and ranges occur in the years of smoking history across three of the four groups. The exception was the control group, which had a noticeably lower mean and range for smoking history, and contained no current smokers. The greatest difference in terms of people who had ever smoked was between those in the placebo and control groups, and this was significant, $\chi^2(1) = 4.25, p = .039$. The next highest difference was between the high GTN and control groups. This approached, but did not attain, statistical significance, $\chi^2(1) = 3.26, p = .071$. An ANOVA comparing the four groups on years of smoking for those who had smoked did not yield a significant $F$ value, but intergroup differences were explored with $t$-tests. The greatest difference in terms of years of smoking, for those who had smoked, was between the high GTN and control groups, and it was statistically significant, $t(15) = 3.21, p = .006$, as was the next greatest difference, namely between the low GTN and control groups, $t(16.89) = 2.95, p = .009$. However, although placebo group members who had ever smoked had done so for slightly fewer years than had members of the two intervention groups, they had not smoked for significantly more years than had those in the control group.
Table 5.6

Demographic Characteristics of Participants in the Four Subgroups (N = 100)\(^a\)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Low GTN dose (n = 33)</th>
<th>High GTN dose (n = 24)</th>
<th>Placebo (n = 22)</th>
<th>Control (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>70 (8.1)</td>
<td>71.7 (8.9)</td>
<td>70.9 (8.0)</td>
<td>68.1 (13.1)</td>
</tr>
<tr>
<td>Minimum–maximum</td>
<td>42–83</td>
<td>52–86</td>
<td>54–83</td>
<td>38–91</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>18 (55)</td>
<td>16 (66)</td>
<td>12 (55)</td>
<td>13 (62)</td>
</tr>
<tr>
<td>Female</td>
<td>15 (46)</td>
<td>8 (33)</td>
<td>10 (45)</td>
<td>8 (38)</td>
</tr>
<tr>
<td>Smoking current</td>
<td>3 (9.1)</td>
<td>1 (4.2)</td>
<td>2 (9.1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Ever-smoked(^b)</td>
<td>15 (45.5)</td>
<td>12 (50)</td>
<td>12 (54.5)</td>
<td>5 (23.8)</td>
</tr>
<tr>
<td>Smoking history years(^b)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>37.1 (20.3)</td>
<td>38.2 (12.7)</td>
<td>32.6 (17.5)</td>
<td>18.2 (–)</td>
</tr>
<tr>
<td>Minimum–maximum</td>
<td>4–60</td>
<td>10–60</td>
<td>1–53</td>
<td>4–25</td>
</tr>
<tr>
<td>High alcohol intake(^c)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcoholic drinks per week(^c)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>35.7 (–)</td>
<td>20 (–)</td>
<td>45.5 (–)</td>
<td>28 (–)</td>
</tr>
<tr>
<td>Minimum–maximum</td>
<td>16–49</td>
<td>20</td>
<td>35–56</td>
<td>28</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>14 (–)</td>
<td>35 (–)</td>
<td>10 (–)</td>
<td>– (–)</td>
</tr>
<tr>
<td>Minimum–maximum</td>
<td>14</td>
<td>35</td>
<td>10–10</td>
<td>–</td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>30.5 (7.2)</td>
<td>30.0 (6.0)</td>
<td>29.2 (5.2)</td>
<td>27.7 (4.0)</td>
</tr>
<tr>
<td>Minimum–maximum</td>
<td>21–50</td>
<td>23–49</td>
<td>19–41</td>
<td>20–34</td>
</tr>
<tr>
<td>Mobility(^d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>6 (18.2)</td>
<td>6 (25.0)</td>
<td>3 (13.6)</td>
<td>2 (9.5)</td>
</tr>
<tr>
<td>High/normal</td>
<td>27 (81.8)</td>
<td>18 (75.0)</td>
<td>19 (86.4)</td>
<td>19 (90.5)</td>
</tr>
</tbody>
</table>

\(^a\) Values are numbers (percentages) unless indicated otherwise. A dash is placed within the parentheses where numbers are too small for standard deviations to be meaningful.

\(^b\) Based on those who have smoked or are currently smoking.

\(^c\) Refers only to men consuming more than 14 standard drinks of alcohol a week (n = 7) and women consuming more than seven standard drinks of alcohol a week (n = 4).

\(^d\) Mobility coded 1. Low: needing assistance with activities of daily living; 2. Medium: impaired but independent with activities of daily living; 3. High/normal: able to engage in discretionary exercise activities.
Higher than recommended alcohol intake existed in each group, but in small numbers. BMI was evenly distributed across the groups with the control group members being slightly lower, and there were higher mobility rates in the placebo and control groups. None of these differences were statistically significant.

Table 5.7 reveals a trend for the high GTN group to contain more pathology. This group had the highest proportion of diabetic participants at almost double that of the control group (70.8% : 42.9%)—although this difference marginally failed to attain statistical significance, $\chi^2(1) = 3.59, p = .058$. The high GTN group also had the highest percentage of participants with other medical conditions (95.8%), but this also marginally failed to reach statistical significance when compared with the control group, which had the lowest percentage (72.6%), $\chi^2(1) = 3.74, p = .053$. Vascular-related conditions were evenly spread across all groups (see Table 5.7).

The entries in Table 5.8 demonstrate fairly even distributions of most of the vascular-related conditions across all four groups apart from the lowest percentages of specific conditions often occurring in the placebo group. The most notable example of this inequity, cardiovascular surgery, had been less frequent in the placebo group (4.5%) compared with the other three groups, but the difference was statistically significant only between the low GTN and placebo groups according to the Fisher’s exact test, $p = .039$. The second most notable feature of this inequity (Table 5.8) is myocardial infarction, which occurred with approximately double frequency in the low GTN group at 18% compared with 9.5% in the control group—a difference that was, however, not statistically significant.

Osteoarthritis in weightbearing or spinal joints was more prevalent at 45.5% in both the low GTN and placebo groups compared with the high dose and control groups at 29.2% and 33.3 % respectively, but none of the differences was statistically significant. Low numbers are present across all groups for the most severe manifestations of lower limb pathology, which are listed as the last four items in Table 5.8: Charcot foot, ulceration, history of ulceration, and amputation.
### Table 5.7
**Health Status of Participants in the Four Subgroups (N = 100)**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Low GTN dose (n = 33)</th>
<th>High GTN dose (n = 24)</th>
<th>Placebo (n = 22)</th>
<th>Control (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic</td>
<td>19 (57.6)</td>
<td>17 (70.8)</td>
<td>13 (59.0)</td>
<td>9 (42.9)</td>
</tr>
<tr>
<td>Diabetes Type 1</td>
<td>4 (12.1)</td>
<td>3 (12.5)</td>
<td>1 (4.5)</td>
<td>1 (4.8)</td>
</tr>
<tr>
<td>Diabetes Type 2</td>
<td>15 (45.5)</td>
<td>14 (58.3)</td>
<td>12 (54.5)</td>
<td>8 (38.1)</td>
</tr>
<tr>
<td>Diabetes: Years since diagnosis, Mean (SD)</td>
<td>17.1 (13.0)</td>
<td>16.0 (14.6)</td>
<td>14.8 (10.1)</td>
<td>19.5 (14.3)</td>
</tr>
<tr>
<td>Diabetes control HbA1c Mean (SD)</td>
<td>6.8 (0.8)</td>
<td>7.6 (1.5)</td>
<td>7.5 (1.3)</td>
<td>7.2 (1)</td>
</tr>
<tr>
<td>With vascular-related condition(s)(^\text{b})</td>
<td>33 (100)</td>
<td>24 (100)</td>
<td>22 (100)</td>
<td>21 (100)</td>
</tr>
<tr>
<td>With other medical condition(s)</td>
<td>31 (93.9)</td>
<td>23 (95.8)</td>
<td>17 (77.3)</td>
<td>16 (76.2)</td>
</tr>
<tr>
<td>Blood pathology liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>20 (60.6)</td>
<td>17 (70.8)</td>
<td>No blood tests in control groups</td>
<td>No blood tests in control groups</td>
</tr>
<tr>
<td>Abnormal</td>
<td>4 (12.1)</td>
<td>2 (8.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No test</td>
<td>9 (27.2)</td>
<td>5 (15.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood pathology kidney</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>17 (51.5)</td>
<td>15 (62.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal</td>
<td>8 (24.2)</td>
<td>4 (16.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No test</td>
<td>8 (24.2)</td>
<td>5 (20.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood pathology FBC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>21 (63.6)</td>
<td>16 (66.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal</td>
<td>5 (15.2)</td>
<td>3 (12.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No test</td>
<td>7 (21.2)</td>
<td>5 (20.8)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\) Values are numbers (percentages) unless indicated otherwise.

\(^{b}\) All participants had at least one vascular-related condition, TBI \(\leq 0.65\) on one or both feet, because that was an inclusion criterion for the study. Most participants had additional vascular conditions. Refer to Table 5.8.
Table 5.8

Vascular-Related Conditions in the Four Subgroups (N = 100)\(^a\)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Low GTN dose (n = 33)</th>
<th>High GTN dose (n = 24)</th>
<th>Placebo (n = 22)</th>
<th>Control (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of vascular-related conditions, Mean (SD)(^b)</td>
<td>2.5 (1.3)</td>
<td>2.4 (1.6)</td>
<td>2.6 (1.5)</td>
<td>2.5 (1.4)</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>6 (18.2)</td>
<td>3 (12.5)</td>
<td>3 (13.6)</td>
<td>2 (9.5)</td>
</tr>
<tr>
<td>Cerebrovascular accident</td>
<td>2 (6.1)</td>
<td>2 (8.3)</td>
<td>2 (9.1)</td>
<td>3 (14.3)</td>
</tr>
<tr>
<td>Carotid artery disease</td>
<td>1 (3)</td>
<td>1 (4.2)</td>
<td>2 (9.1)</td>
<td>1 (4.8)</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>4 (12.1)</td>
<td>2 (8.3)</td>
<td>2 (9.1)</td>
<td>1 (4.8)</td>
</tr>
<tr>
<td>Lower limb vascular surgery</td>
<td>4 (12.1)</td>
<td>3 (12.5)</td>
<td>1 (4.5)</td>
<td>4 (19)</td>
</tr>
<tr>
<td>Cardiovascular surgery</td>
<td>9 (27.3)</td>
<td>6 (25)</td>
<td>1 (4.5)</td>
<td>4 (19)</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>0 (0)</td>
<td>1 (4.2)</td>
<td>1 (4.5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Osteoarthritis</td>
<td>15 (45.5)</td>
<td>7 (29.2)</td>
<td>10 (45.5)</td>
<td>7 (33.3)</td>
</tr>
<tr>
<td>Connective tissue disease</td>
<td>1 (3)</td>
<td>0 (0)</td>
<td>1 (4.8)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Raynaud’s disease</td>
<td>3 (9.1)</td>
<td>1 (4.2)</td>
<td>4 (18.2)</td>
<td>4 (19)</td>
</tr>
<tr>
<td>Chilblains</td>
<td>2 (6.1)</td>
<td>3 (12.5)</td>
<td>3 (13.6)</td>
<td>5 (23.8)</td>
</tr>
<tr>
<td>Charcot foot</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (4.8)</td>
</tr>
<tr>
<td>Foot ulcer</td>
<td>1 (3)</td>
<td>3 (12.5)</td>
<td>0 (0)</td>
<td>1 (4.8)</td>
</tr>
<tr>
<td>History of foot ulcer</td>
<td>2 (6.1)</td>
<td>2 (8.3)</td>
<td>1 (4.5)</td>
<td>1 (4.8)</td>
</tr>
<tr>
<td>Amputation</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (9.1)</td>
<td>1 (4.8)</td>
</tr>
</tbody>
</table>

\(^a\) Values are numbers (percentages) unless indicated otherwise.

\(^b\) Entries in this top row represent low TBIs as one cardiovascular related condition for all participants as well as any of the additional conditions listed in this table.
Table 5.9 presents the medications taken by participants in the four groups. The following features are noteworthy:

- Antihypertensives peaked in the high GTN group at 91.7% and were distributed with a 30% difference in spread across the other groups to a minimum of 61.9% in the control group. The only statistically significant difference was that between the high GTN and control groups according to the Fisher’s exact test, \( p = .029 \).

- Hypolipidaemic medications were spread across the groups with a slight peak in the placebo group, and none of the differences were statistically significant.

- The four groups were similar in the use of antiarrythmic medications, with very small numbers in each group.

- Antigoagulants (including aspirin) were spread unevenly across the four groups with the largest difference being 17% between the low GTN group at 51% and the placebo group at 68%. Chi square analysis indicated that this difference was not statistically significant.

- The antianginal medications occur almost exclusively in the low GTN group at a prevalence of 18%. This was not significantly greater than was the use of antianginal medication in any of the other groups.

- Insulin use is closely matched within 4.6% across the four groups.

- Lowest use of oral antihyperglycemics occurred in the control group at 23.8%, but not to a significantly lower extent than in the high GTN or placebo groups, both at 50% usage, with outcomes of \( \chi^2(1) = 3.27, p = .071 \) and \( \chi^2(1) = 3.15, p = .076 \) respectively. The percentage of participants taking other medications was quite closely matched across all four groups.

- The number of other medications was highest in the in the low GTN group, and lowest in the control group. Although an ANOVA comparing all four groups did not yield a significant \( F \) value, the difference between low GTN and control groups was significant, \( t(44) = 2.45, p = .018 \). No other comparisons were significant.
Table 5.9

Medications of Participants in the Four Subgroups (N = 100)\(^a\)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Low GTN dose (n = 33)</th>
<th>High GTN dose (n = 24)</th>
<th>Placebo (n = 22)</th>
<th>Control (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antihypertensives</td>
<td>26 (78.8)</td>
<td>22 (91.7)</td>
<td>18 (81.8)</td>
<td>13 (61.9)</td>
</tr>
<tr>
<td>Hypolipidemics</td>
<td>22 (66.7)</td>
<td>15 (62.5)</td>
<td>17 (77.3)</td>
<td>11 (52.4)</td>
</tr>
<tr>
<td>Antiarrythmics</td>
<td>0 (0)</td>
<td>2 (8.3)</td>
<td>1 (4.5)</td>
<td>1 (4.8)</td>
</tr>
<tr>
<td>Anticoagulants(^b)</td>
<td>17 (51.5)</td>
<td>15 (62.5)</td>
<td>15 (68.2)</td>
<td>12 (57.1)</td>
</tr>
<tr>
<td>Antianginals</td>
<td>6 (18.2)</td>
<td>0 (0)</td>
<td>1 (4.5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Insulin</td>
<td>9 (27.3)</td>
<td>6 (25)</td>
<td>5 (22.7)</td>
<td>6 (28.6)</td>
</tr>
<tr>
<td>Oral antihyperglycaemcs</td>
<td>14 (42.4)</td>
<td>12 (50)</td>
<td>11 (50)</td>
<td>5 (23.8)</td>
</tr>
<tr>
<td>Number taking other</td>
<td>28 (84.8)</td>
<td>21 (87.5)</td>
<td>18 (81.8)</td>
<td>18 (85.7)</td>
</tr>
<tr>
<td>medications(^c), mean (SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimum–maximum</td>
<td>4.1 (2.6)</td>
<td>2.6 (1.7)</td>
<td>3.2 (2.5)</td>
<td>2.4 (1.9)</td>
</tr>
</tbody>
</table>

\(^a\) Values are numbers (percentages) unless indicated otherwise.

\(^b\) Includes warfarin, platelet inhibitors, NSAIDs, and heparin.

\(^c\) Based only on medications additional to those listed above.

5.3.1.1.3 Summary of participant characteristics at baseline

The study population, being a group distinguished by the presence of PAD was different from the general population in some health characteristics such as higher than average BMI.

Smoking rates within the whole sample are lower than those in the general population. However, 44% of the participants were ex-smokers. Similarly, the good levels of diabetic control as determined by the ideal HbA1c, although desirable, are not typical of the diabetic population in general (Speight, 2013).

Although the four groups were similar in the domains of age as well as the prevalence, severity, and duration of diabetes, there were differences on other variables.
Table 5.10 provides a summary of the statistically significant differences and their \( p \) values. Among those that were statistically significant, there was a greater percentage of participants currently smoking, and there were more years of smoking history, in the intervention groups compared with the controls. There were also more people with medical conditions in the intervention groups relative to the nonintervention groups and there had been more cardiovascular surgery in the low GTN group than in the placebo group. There was also greater hypertension and medication within at least one of the intervention groups compared with either the placebo or control groups, indicating that the latter groups had less medicated pathology.

### Table 5.10

**Group Differences That Were Statistically Significant**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Higher group</th>
<th>Lower group</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ever smoked</td>
<td>Placebo</td>
<td>Control</td>
<td>.039</td>
</tr>
<tr>
<td>Years of smoking</td>
<td>High GTN</td>
<td>Control</td>
<td>.006</td>
</tr>
<tr>
<td>Years of smoking</td>
<td>Low GTN</td>
<td>Control</td>
<td>.009</td>
</tr>
<tr>
<td>Additional medical conditions</td>
<td>Intervention</td>
<td>Nonintervention</td>
<td>.008</td>
</tr>
<tr>
<td>Cardiovascular surgery</td>
<td>Low GTN</td>
<td>Placebo</td>
<td>.039</td>
</tr>
<tr>
<td>Antihypertensives</td>
<td>High GTN</td>
<td>Control</td>
<td>.029</td>
</tr>
<tr>
<td>Number of other medications</td>
<td>Low GTN</td>
<td>Control</td>
<td>.018</td>
</tr>
</tbody>
</table>

Table 5.11 provides a summary of the differences that approached but did not attain statistical significance. Again, it is evident that the control group could be regarded as healthier, particularly with regard to the high GTN group. On balance, the demographic and medical breakdowns indicate that on most variables the groups were similar to each other, but that when differences occurred, compared with the intervention groups the placebo and control groups were generally healthier and had less pathology.
Table 5.11  
*Group Differences That Approached Statistical Significance*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Higher group</th>
<th>Lower group</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ever smoked</td>
<td>High GTN</td>
<td>Control</td>
<td>.071</td>
</tr>
<tr>
<td>Diabetic</td>
<td>High GTN</td>
<td>Control</td>
<td>.058</td>
</tr>
<tr>
<td>Other medical conditions</td>
<td>High GTN</td>
<td>Control</td>
<td>.053</td>
</tr>
<tr>
<td>Cardiovascular surgery</td>
<td>Intervention</td>
<td>Nonintervention</td>
<td>.069</td>
</tr>
<tr>
<td>Oral antihyperglycemics</td>
<td>High GTN</td>
<td>Control</td>
<td>.071</td>
</tr>
<tr>
<td>Oral antihyperglycemics</td>
<td>Placebo</td>
<td>Control</td>
<td>.076</td>
</tr>
</tbody>
</table>

5.3.1.2 Participants lost to follow-up / rejected from analysis

A total of 22 participants (17.9%) who were initially recruited to the study were subsequently either lost to follow-up (n = 21) or not included in the final analyses because of lack of compliance (n = 1). Among these, as indicated in the participant flowchart in Section 5.3.1, eight were in the low GTN group, seven in the high GTN group, three in the placebo group, and four in the control group, representing 20%, 22%, 12%, and 18% respectively from these groups’ original numbers. Refer to Table K.1 in Appendix K.

As can be seen in Table K.2 in Appendix K, the most frequent issue for withdrawal occurred in five people (23%) who discontinued because of medical conditions directly associated with CVD. An additional three (14%) suffered from exacerbation of concomitant chronic illnesses. Scheduling and transport logistics proved a barrier to three more people (14%). Two (9%) experienced acute mobility-disrupting lower limb injuries resulting from falls, and for another two people advanced age was provided as the barrier that precluded their ongoing participation. Two were offered vascular specialist intervention and preferred to proceed with that process for their symptomatic PAD. Four people (18%) withdrew without providing specific reasons. Finally, one person disclosed that he had been experimenting with his GTN patches, applying them to both feet, and had not used his patches at all while on holidays for 4 months. This person was suffering with painful diabetic neuropathy. After some initial pain relief,
GTN appeared ineffective for his ongoing pain management. This frustration drove his experimentation. Finding a solution was his motivation that overrode his commitment to study compliance. He was the only person who attended for all visits but was excluded from the analyses because of noncompliance.

As can also be seen in Table K.2 (Appendix K), none of the above reasons was noticeably associated with any single one of the four groups of participants, and most people who withdrew did so before the second or third visit. Two who discontinued later, at 4 and 5 months, were both control group people who became unwell due to their preexisting chronic poor health status. None of the people who discontinued from the two intervention groups did so for reasons that appeared to be associated with adverse side effects. Two of the four people in the control group gave no reason for discontinuing, and this was the greatest in both number and proportion of any group for those who discontinued without a specific reason. Given the above analyses, withdrawal of participants was unlikely to have affected the results.

5.3.2 Description and validity of initial TBI readings

Exploration of TBI readings commenced by making decisions about management of the most extreme outliers in the data. Detailed analyses were then performed on the TBI readings to determine their distributions and validity and whether cuff size affected toe pressures. These preliminary processes formed a necessary platform for the subsequent analyses.

Five initial adjustments were made within the 3,650 TBI readings because the original readings were very high and clearly spurious. These adjustments are shown in Table 5.12 along with relevant explanations. Because only the highest outliers were addressed at this stage, the data still contained outliers, inliers that were likely to be erroneous, and highly variable readings that were all later addressed.
Table 5.12

Initial Adjustments of Data to Remove Extreme Outliers

<table>
<thead>
<tr>
<th>Participant number</th>
<th>Original score</th>
<th>Altered score</th>
<th>Reason for alteration / action</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>1.38</td>
<td>1.07</td>
<td>Given all the other readings for this person, the TBI of 1.38 appears to be a misreporting, so this reading was converted to 1.07 because the other two readings of that visit on that foot were both 1.07.</td>
</tr>
<tr>
<td>11</td>
<td>1.41</td>
<td>.57</td>
<td>Given all the other recordings for this person, this appears to be a very bad misreading, or an extremely unusual reading, so this was converted to 0.57 because that was the average of the other two readings from that foot on this visit.</td>
</tr>
<tr>
<td>44</td>
<td>2.165</td>
<td>.93</td>
<td>Clearly this was an error, so that day’s third reading was substituted for it because it was more typical of readings on that foot on most visits for this control group participant.</td>
</tr>
<tr>
<td>47</td>
<td>1.99</td>
<td>.41</td>
<td>This was clearly an error, so that day’s first and third readings were averaged.</td>
</tr>
<tr>
<td>70</td>
<td>1.41</td>
<td>.23</td>
<td>This high outlier appears to be a misreading given that all of the other readings for this person were around 0.30 or lower. The other two readings from that foot for that day were averaged.</td>
</tr>
</tbody>
</table>

5.3.2.1 Description of initial TBI readings

In the two subsections that follow, the initial TBI readings obtained at baseline (after adjusting the extreme outliers; refer to previous section) are described—first on the basis of a single reading for each foot (i.e., the average of the second and third readings), and then in terms of each of the three readings taken on each foot. These baseline readings were considered most representative of actual toe pressures of this sample because readings on visits subsequent to baseline could have been influenced by GTN treatment, or placebo, effects. It is arguable that these data also represent readings that would be typically obtained and used in clinical and research situations with similar populations.
5.3.2.1.1 Distributions of single readings for each foot

In order to have a single reading for each foot, the recommendation of Pérez-Martin et al. (2010) to take the average of the second and third of three readings was adopted. These TBIs are provided in Table 5.13 and Figure 5.2.

Table 5.13

<table>
<thead>
<tr>
<th>Statistic</th>
<th>Total sample (N =97)</th>
<th>Low GTN dose (n =32)</th>
<th>High GTN dose (n = 22)</th>
<th>Placebo (n = 22)</th>
<th>Control (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foot</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LbTBI</td>
<td>.575 (.169)</td>
<td>.566 (.160)</td>
<td>.592 (.164)</td>
<td>.540 (.153)</td>
<td>.608 (.203)</td>
</tr>
<tr>
<td>HbTBI</td>
<td>.690 (.189)</td>
<td>.702 (.198)</td>
<td>.680 (.166)</td>
<td>.683 (.207)</td>
<td>.690 (.189)</td>
</tr>
<tr>
<td>Min.–max.</td>
<td>.23–1.13</td>
<td>.23–.92</td>
<td>.24–.88</td>
<td>.30–.78</td>
<td>29–1.13</td>
</tr>
<tr>
<td>HbTBI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Values are means (SDs). Data are based on the average of the second and third TBI readings after five extreme outliers had been adjusted but prior to subsequent data cleaning.

b Three participants had missing data due to cold skin temperatures, so an average value could not be calculated in those cases.

Figure 5.2. Histograms of the average of second and third TBI readings on each foot at baseline.
Although the data were subsequently adjusted to increase validity, it is evident that, as expected from the group allocation process, the mean of the LbTBI foot (0.575) was lower than that of the HbTBI foot (0.689). This difference was statistically significant, $t(192) = 4.48, p < .001$. The direction of this difference was maintained across all four subgroups. Furthermore, for each foot across all subgroups the mean TBIs were similar to each other, so at this preliminary stage the nonrandom group allocation process appears not to have resulted in between-group differences. In addition, the Levene test indicated that there was equality of variance across the groups, $F = 0.894, p = .345$. There was, however, a noticeably greater spread of scores in the placebo group (see Table 5.13). Particularly evident was its maximum score being well above the other groups’ maximum scores.

This analysis was revised after further management of outliers and anomalous TBI readings, and after adjustments had been made to take the effect of cuff size into account. Details concerning this are presented in Section 5.3.4.3.

The histograms in Figure 5.2 reveal that there is an approximately normal distribution in the readings from both feet, but that some high outliers are still present in the data, with maximums of 1.13 on the LBTBI foot and 1.26 on the HBTBI foot.

5.3.2.1.2 Distributions of first, second, and third TBI readings

Descriptive statistics for the three separate readings on each foot at baseline are provided in Table 5.14. Consistent with the results in the previous section, and as expected, the difference between the lower and higher pressure feet is maintained across each of the three sets of readings. For example, the mean of the first reading on the LbTBI foot was 0.557, whereas the mean of the first reading on the HbTBI foot was 0.678. Furthermore, with the exception of the first reading where the minimum value is equal for both feet, the minimum and maximum values of the LbTBI foot are lower than those of the HbTBI foot.
Although dealt with in more detail in the next section, it is obvious from entries in Table 5.14 that, for each foot, on average the first reading was slightly lower than the other two readings, and its standard deviation was slightly larger.

Table 5.14

<table>
<thead>
<tr>
<th>Foot</th>
<th>First reading</th>
<th>Second reading</th>
<th>Third reading</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>LbTBI</td>
<td>.557</td>
<td>.183</td>
<td>.571</td>
</tr>
<tr>
<td>Minimum–maximum</td>
<td>.19–1.12</td>
<td></td>
<td>.23–1.13</td>
</tr>
<tr>
<td>HbTBI</td>
<td>.678</td>
<td>.220</td>
<td>.697</td>
</tr>
<tr>
<td>Minimum–maximum</td>
<td>.19–1.40</td>
<td></td>
<td>.27–1.23</td>
</tr>
</tbody>
</table>

Entries are based on readings from 100 participants (200 feet).

The distributions relevant to Table 5.14 are depicted in Figure 5.3 (three histograms) and Figure 5.4 (also three histograms). The highest readings in almost every histogram are discontinuous with other data, indicating some extreme outliers. The data do not seem to be skewed, platykurtic, or leptokurtic, however, and most distributions have a wide spread with distributions approaching normality. The most noticeable exceptions are outliers of 1.40 and 1.29 in the HbTBI foot readings (see Figure 5.4). Clearly the averaging procedure used in the previous section had attenuated the extent of these outliers.
Figure 5.3. Histograms of first, second, and third TBI readings on LbTBI foot at baseline.
Figure 5.4. Histograms of first, second, and third TBI readings on HbTBI foot at baseline.
5.3.2.2 Comparisons among first, second, and third TBI readings

Testing for differences between the first, second, and third TBI readings was conducted to help ascertain which of the readings, or which combination of readings, would be the most valid to use for analyses. Two issues are addressed in this section:

The direction of differences between the three readings, in particular whether the first reading was significantly and consistently lower than the other two (refer to Section 5.3.2.2.1), and, the extent to which the readings differed from each other (refer to Section 5.3.2.2.2).

5.3.2.2.1 Direction of differences between first, second, and third TBI readings

The direction of differences between the three TBI readings was explored by using three sets of approximately 1,210 pairs of observations because the focus was on comparisons between readings, not on TBIs for individual participants. In doing this, the prospect of nonindependence of measurements is acknowledged (see Menz, 2004).

The data in Table 5.15 indicate that, despite expectations, the first reading was not consistently lower than the second and third readings. In fact, in 42.8% of cases the first reading was higher than the second, and in 44.4% of cases it was higher than the third. Furthermore, the second reading was not consistently either higher or lower than the third, the second being higher than the third 44.4% of the time and lower 46.9% of the time. Overall, therefore, the order of the readings did not generate consistent differences between the values of the readings.

Table 5.15

*Frequency with which First, Second, and Third TBI Readings are Higher or Lower than Each Other, or Equivalent*

<table>
<thead>
<tr>
<th>Comparison of readings</th>
<th>Number of readings</th>
<th>First reading greater than second</th>
<th>No difference between readings</th>
<th>Second reading greater than first</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>1 vs 2</td>
<td>1,214</td>
<td>520</td>
<td>42.8</td>
<td>74</td>
</tr>
<tr>
<td>1 vs 3</td>
<td>1,210</td>
<td>537</td>
<td>44.4</td>
<td>65</td>
</tr>
<tr>
<td>2 vs 3</td>
<td>1,211</td>
<td>542</td>
<td>44.8</td>
<td>101</td>
</tr>
</tbody>
</table>

*a* Analyses are based on both feet across six visits with some additional data from Visit 2.
5.3.2.2 Extent of differences between first, second, and third TBI readings

Two sets of analyses were performed to examine the extent of differences between the three readings. In the first set of analyses, all readings across the trial period were included because, as in the previous subsection, the focus could be regarded as being on comparisons between readings, not on TBIs for participants. Thus, the prospect of nonindependence of data is again acknowledged. A subsequent set of analyses based on independence of data from each foot at baseline, Visit 3, and Visit 7 was performed in case different results might have been produced under those circumstances. These two different sets of analyses are described in the next two subsections.

Readings based on both feet across all visits. For the analyses using all available TBI readings (and therefore nonindependence of data), raw data were initially used, and, for each set of three readings, differences were calculated between the values obtained for Reading 1 versus Reading 2, Reading 1 versus Reading 3, and Reading 2 versus Reading 3. The results are shown in the Raw differences column of Table 5.16. The means of these raw differences are uniformly very small, ranging from only –0.011 to 0.002. These means could be regarded as indicating that the differences between the three readings for any one set of readings were negligible. However, both the size of the standard deviations in Table 5.13 and visual inspection of the raw data themselves indicate that there were often noticeable discrepancies. This is also evident in the Largest differences column of Table 5.16, where there are discrepancies as large as 1.19 and 1.16 between some pairs of readings. The means based on the raw data therefore do not provide an indication of the size of the discrepancies within each set of three readings that are present in the data. Those means simply indicate that there are approximately equally sized positive and negative deviations from the means, each cancelling the other out.

As another, more revealing, approach for ascertaining the extent of differences between readings, absolute values of differences between them were calculated. Table 5.16 includes entries that are based on those absolute values. They reveal that the biggest discrepancies occur between Readings 1 and 3 (average discrepancy of 0.092), followed closely by the discrepancies between Readings 1 and 2 (average discrepancy of 0.084). Readings 2 and 3 differed least (average discrepancy of 0.065). These
average discrepancies are not large, whether they are the smallest one associated with Readings 2 and 3, or the larger ones associated with the other two sets of comparisons.

Table 5.16

*Extent of Differences Between First, Second, and Third TBI Readings*\(^a\)

<table>
<thead>
<tr>
<th>Comparison of readings</th>
<th>Number of readings</th>
<th>Raw differences</th>
<th>Absolute differences</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>Mean</td>
</tr>
<tr>
<td>1 vs 2</td>
<td>1,214</td>
<td>-.010</td>
<td>.084</td>
</tr>
<tr>
<td>1 vs 3</td>
<td>1,210</td>
<td>-.011</td>
<td>.092</td>
</tr>
<tr>
<td>2 vs 3</td>
<td>1,211</td>
<td>.002</td>
<td>.065</td>
</tr>
</tbody>
</table>

\(^a\) Analyses are based on both feet across six visits (with some additional data from Visit 2)

Analysis of quartile splits in the data also indicates that most sets of readings were similar to each other. Across the total number of comparisons (i.e., approximately 1,200 for each pair of comparisons), in 75% of cases the absolute difference between Readings 1 and 2 was less than 0.11, in 75% of the comparisons between Readings 1 and 3 the absolute difference was less than 0.12, and in 75% of the comparisons between Readings 2 and 3 the difference was less than 0.08. Again, Readings 2 and 3 were closer to each other, but not to a large extent.

Although the average differences between the three comparisons are similar to each other (ranging from only 0.065 to 0.092), the standard deviations indicate that on a number of occasions two of the three readings differed from each other by more than 0.10. Further analysis indicates that an absolute difference greater than 0.20 occurred in approximately 7% of the comparisons between Readings 1 and 2, 10% of the comparisons between Readings 1 and 3, and 5% of the comparisons between Readings 2 and 3. The extent of these differences and the size of the maximum differences in the final column of Table 5.16 (i.e., 1.19, 1.16, and 0.84 for the three sets of comparisons respectively) indicate that careful detection and inspection of anomalous recordings would be justified—certainly for the present data set.
Readings on separate feet at baseline, Visit 3, and Visit 7. Analyses in this section parallel those in the previous section (with the exception of the minimally informative raw data analyses), with the intention of confirming or disconfirming the results obtained there when nonindependence of measurements was regarded as acceptable. Here independence of measurements is observed, with each foot being analysed separately at baseline, Visit 3, and Visit 7. The results from the baseline and Visit 3 analyses are summarised in Table 5.17. Results from the Visit 7 analysis are not presented in that table, but are discussed within the text below.

As can be seen from entries in Table 5.17, at baseline, on the LbTBI foot the smaller discrepancy in the comparison between Readings 2 and 3, as opposed to the comparisons involving Readings 1 and 2, and 1 and 3 (differences of 0.054, 0.072, and 0.073 respectively), is again apparent as it was when all readings were analysed together. However, the differences remain small, (ranging from only 0.054 to 0.073), so the three readings seem to be similar in terms of the average extent to which they differ from each other. Average discrepancies among readings on the HbTBI foot were only slightly larger (ranging from 0.066 to 0.095), but again they were all relatively similar to each other.
As can be seen from entries in Table 5.17, at Visit 3, for the LbTBI foot the greater similarity between Readings 2 and 3, as opposed to the comparisons between Readings 1 and 2, and between Readings 1 and 3 (differences ranging from 0.069 to 0.100), is again apparent, but the readings are a little more discrepant than they were at baseline. Results on the HbTBI foot at Visit 3 are similar: greater similarity between Readings 2 and 3, and none very different from the others (ranging from 0.061 to 0.097). These results are similar to those obtained at baseline.

At Visit 7, the same pattern emerged. For the LbTBI foot, the greater similarity between Readings 2 and 3, as opposed to the comparisons between Readings 1 and 2, and between Readings 1 and 3 (differences of 0.059, 0.088, and 0.090 respectively), was evident once more, with the range of difference between the means again being small. For the HbTBI foot, the differences between Readings 2 and 3, Readings 1 and 2, and Readings 1 and 3 were 0.069, 0.081, and 0.089 respectively, so again there was greater similarity between Readings 2 and 3 than between the other two comparisons but, as previously, the average difference between the three pairs of readings was small.
Overall, the above analyses indicate that although the means of the first, second, and third TBI readings were different from each other, those differences were very small (see Table 5.14). Furthermore, across each set of three TBI readings there were no systematic or large differences in one or the other direction between the three readings.

5.3.2.3  Correlations between first, second, and third TBI readings

As another means of exploring the set of three readings, correlations between them at baseline, Visit 3, and Visit 7 were calculated using the data from all participants. The results are shown in Table 5.18. Spearman’s rank order correlation coefficients were used because of outliers that had still been retained in the data.

Table 5.18

<table>
<thead>
<tr>
<th></th>
<th>Reading 1</th>
<th>Reading 2</th>
<th>Reading 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reading 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td>.83</td>
<td>.79</td>
</tr>
<tr>
<td>Visit 3</td>
<td>.85</td>
<td></td>
<td>.83</td>
</tr>
<tr>
<td>Visit 7</td>
<td>.84</td>
<td>.85</td>
<td></td>
</tr>
<tr>
<td>Reading 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>.79</td>
<td></td>
<td>.85</td>
</tr>
<tr>
<td>Visit 3</td>
<td>.86</td>
<td></td>
<td>.90</td>
</tr>
<tr>
<td>Visit 7</td>
<td>.91</td>
<td></td>
<td>.92</td>
</tr>
<tr>
<td>Reading 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td>.82</td>
<td>.84</td>
</tr>
<tr>
<td>Visit 3</td>
<td>.83</td>
<td></td>
<td>.90</td>
</tr>
<tr>
<td>Visit 7</td>
<td>.88</td>
<td></td>
<td>.93</td>
</tr>
</tbody>
</table>

* Correlations are Spearman’s rank order coefficients. Entries above the diagonal refer to the LbTBI foot; those below the diagonal refer to the HbTBI foot. \( N \geq 97 \) for all entries, and all are significant at the < .001 level.

These correlations indicate that the associations between the three readings are consistently strong and generally become slightly stronger from baseline to Visit 7. They also confirm that Readings 2 and 3 were always, but not usually remarkably,
closer than were either Readings 1 and 2 or Readings 1 and 3. The lack of perfect correlation indicates some degree of discrepancy among the readings, however.

Intraclass correlation coefficients (ICCs)\(^7\) between each pair of readings were also obtained at baseline, Visit 3, and Visit 7 as an additional means of assessing the extent of association between the three readings. The results are shown in Table 5.19. In combination with the high correlation coefficients between readings in Table 5.18, the high ICCs in Table 5.19 suggest that the three readings are generally very similar to each other. However, there is continuing evidence of greater similarity between Readings 2 and 3 than between the other two sets of comparisons, and again the lack of extremely high correlations suggests a degree of disparity in some places within the data.

Table 5.19

*Intraclass Correlations Between First, Second, and Third TBI Readings at Baseline, Visit 3, and Visit 7*\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>Reading 1</th>
<th>Reading 2</th>
<th>Reading 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reading 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>.84</td>
<td>.79</td>
<td></td>
</tr>
<tr>
<td>Visit 3</td>
<td>.86</td>
<td>.82</td>
<td></td>
</tr>
<tr>
<td>Visit 7</td>
<td>.84</td>
<td>.84</td>
<td></td>
</tr>
<tr>
<td>Reading 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>.80</td>
<td>.89</td>
<td></td>
</tr>
<tr>
<td>Visit 3</td>
<td>.85</td>
<td>.92</td>
<td></td>
</tr>
<tr>
<td>Visit 7</td>
<td>.90</td>
<td>.93</td>
<td></td>
</tr>
<tr>
<td>Reading 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>.79</td>
<td>.86</td>
<td></td>
</tr>
<tr>
<td>Visit 3</td>
<td>.81</td>
<td>.91</td>
<td></td>
</tr>
<tr>
<td>Visit 7</td>
<td>.88</td>
<td>.92</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Entries above the diagonal refer to the LbTBI foot; those below the diagonal refer to the HbTBI foot. \(N \geq 97\) for all entries.

\(^7\) All intraclass correlation coefficients in this research are (3,1) with absolute agreement. An ICC greater than .75 is usually considered adequate as an indication of a significant relationship between two variables (Portney & Watkins, 2009, p. 594).
5.3.2.4 Relationship of cuff size to TBIs

The possibility that small cuffs would yield both higher pressures and greater variability in values compared with large cuffs was raised within the minimal literature currently available about cuff size influences (Påhlsson et al., 2007). Entries in Table 5.20 indicate that small cuffs were used with almost half of the total sample but that there were different proportions in each subgroup. Therefore, the placebo group (with 63.6% using small cuffs) could contain more artificially inflated and variable readings relative to the other groups and, conversely, the control group (23.8% with small cuffs) could present fewer of these inflated and variable readings. The prospect that small cuffs produced higher and more varied readings therefore required examination.

<table>
<thead>
<tr>
<th>Cuff size</th>
<th>Total sample</th>
<th>Low GTN dose</th>
<th>High GTN dose</th>
<th>Placebo</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total sample</td>
<td>Low GTN dose</td>
<td>High GTN dose</td>
<td>Placebo</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>n = 100</td>
<td>n = 33</td>
<td>n = 24</td>
<td>n = 22</td>
<td>n = 21</td>
</tr>
<tr>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Small</td>
<td>46 46.0</td>
<td>17 51.5</td>
<td>10 41.7</td>
<td>14 63.6</td>
<td>5 23.8</td>
</tr>
<tr>
<td>Large</td>
<td>54 54.0</td>
<td>16 48.5</td>
<td>14 58.3</td>
<td>8 36.4</td>
<td>16 76.2</td>
</tr>
</tbody>
</table>

In order to determine whether participants with whom small cuffs were used had higher and more variable readings than did those with whom large cuffs were used, three analyses using Mann-Whitney U tests on the average of the second and third TBIs were conducted. The first of these was based on both feet across all visits ($N \approx 1,200$), and therefore the data were not independent; the other two analyses were conducted on each foot separately at baseline, and therefore involved independently observed data.

All analyses indicated that smaller cuff sizes yielded significantly higher TBIs. In the first analysis (nonindependent data), ranks for the small and large cuffs on both feet across all visits were 723.7 and 500.3 respectively, $z = 11.10, p < .001$. For the analyses involving independence of observations, ranks for the small and large cuffs on the LbTBI foot at baseline were 56.91 and 42.7 respectively, $z = 2.47, p = .014$, and for the
HbTBI foot at baseline, ranks for the small and large cuffs were 59.97 and 39.90 respectively, $z = 3.50, p < .001$.

As a follow-up to these analyses, baseline readings of each foot from each person were again analysed separately to ensure independence of observations. However, this time independent samples $t$-tests were used. Use of $t$-tests was considered permissible because the data contained few outliers and $t$-tests are reasonably robust despite their presence. Furthermore, $t$-tests have the advantage of producing (informative) means and standard deviations, as well as results for the Levene test for equality of variance, as part of their output. Table 5.21 contains the means and standard deviations of the first, second, and third readings for the LbTBI and HbTBI feet, for both small and large cuffs, as well as $t$-test and Levene test results.

Table 5.21

<table>
<thead>
<tr>
<th>Reading</th>
<th>Foot</th>
<th>Mean</th>
<th>SD</th>
<th>Mean</th>
<th>SD</th>
<th>$t$</th>
<th>df</th>
<th>Sig.</th>
<th>$F$</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>LbTBI</td>
<td>.593</td>
<td>.20</td>
<td>.528</td>
<td>.17</td>
<td>1.79</td>
<td>97</td>
<td>.077</td>
<td>.066</td>
<td>.798</td>
</tr>
<tr>
<td></td>
<td>HbTBI</td>
<td>.762</td>
<td>.23</td>
<td>.605</td>
<td>.18</td>
<td>3.82</td>
<td>97</td>
<td>&lt; .001</td>
<td>3.168</td>
<td>.078</td>
</tr>
<tr>
<td>Second</td>
<td>LbTBI</td>
<td>.612</td>
<td>.19</td>
<td>.535</td>
<td>.16</td>
<td>2.28</td>
<td>98</td>
<td>.025</td>
<td>.012</td>
<td>.915</td>
</tr>
<tr>
<td></td>
<td>HbTBI</td>
<td>.769</td>
<td>.19</td>
<td>.632</td>
<td>.19</td>
<td>3.61</td>
<td>97</td>
<td>&lt; .001</td>
<td>.008</td>
<td>.927</td>
</tr>
</tbody>
</table>

The $t$-test entries in Table 5.21 indicate that for five of the six sets of readings, the small cuffs were associated with significantly higher TBIs than were the large cuffs. The exception was that for the LbTBI foot the difference between small and large cuffs was not statistically significant based on a two-tailed test, $p = .067$. However, the $t$ value of 1.85 is significant at the .05 level for a one-tailed test, and, because there was an a priori expectation that small cuffs would produce higher TBI readings, this $t$ value might also be regarded as significant. Overall, therefore, the small cuffs produced significantly higher TBI readings.
According to the Levene test, none of the six analyses summarised in Table 5.21 indicate a difference in the variances between the small and large cuff readings. However, for the first reading on the untreated foot, the Levene test approached statistical significance with a 2-tailed $p$ value of .078, suggesting that there was greater variability associated with the small cuff relative to the large cuff. This variability is reflected in the standard deviations of .23 and .18 relating to the first reading for the small and large cuffs respectively (see second row of entries in Table 5.21). Overall, therefore, the large and small cuffs seem to produce similar amounts of variability.

5.3.2.5 Summary regarding description and validity of initial TBI readings

Initial inspection of the data indicated that the allocation of participants to the four subgroups had resulted in an acceptable, even arguably high, degree of similarity regarding TBI scores. However, some spurious data points required immediate adjustment, and there was evidence that additional data, particularly high outliers, would need subsequent attention, possibly involving adjustment or even removal—issues that are dealt with in the next section.

A variety of analyses from the three different data collection points (baseline, Visit 3, and Visit 7), based on both nonindependence and independence of observations, as well as assessments of difference, variation, and correlation, indicate that for the most part the three TBI readings were similar to each other. However, on up to 10% of occasions discrepant measures occurred. These were sufficiently large in some instances that subsequent investigation of them would seem necessary to ensure validity of the data.

Although small and large cuffs produced similar amounts of variability within the TBI readings, small cuffs were responsible for significantly higher readings, and that also would need to be taken into consideration to make valid comparisons between participants and groups.
5.3.3 Adjustments to TBI data to improve validity

Given the findings obtained throughout the previous section (i.e., Section 5.3.2), any one of the three readings could be as valid as the other two. Therefore, the recommendation of Pérez-Martin et al. (2010) to average the second and third measures was abandoned. Instead, the lowest reading of the three taken at any point in time was assumed to be the one least influenced by inflating artifacts and was chosen as the single data value for further analysis. As a result, the data for baseline, Visit 3, and Visit 7—the main focal points of this research—were re-entered into the SPSS data file using the lowest TBI value for each set of three readings.

Following that, a scan of the entire data set was made to identify obvious outliers as well as unusual patterns in the readings that might reveal either erroneous inliers or atypical data points. Potentially problematic or unusual data were identified in one of three ways. First, box plots, histograms, and lists of frequencies were inspected for outliers. Values > 1.20 were immediately regarded as outliers (see Høyer et al., 2013). Second, readings that were more than 0.20 different from either of the other two readings for a particular foot on a particular day were flagged as possible erroneous inliers. Third, the complete data set was scanned for patterns of readings that were conspicuously unusual. The figure of 0.20 represents 2.5 SDs, rounded to the nearest decimal point, based on the average of the three SDs in Table 5.16.

Any data entries identified as possibly problematic were compared with the same person’s data across both feet at all visits to determine whether there might be any justification for altering the original reading to something that would have been more typical of that person’s TBI for that foot on that day. As a result of this process, 17 alterations were made to the data—one alteration for 10 participants, two alterations for two participants, and three alterations for one participant. Details of these alterations are shown in Tables 5.22, 5.23, and 5.24. Given that 600 data points were involved (two feet for 100 people at baseline, Visit 3, and Visit 7), this represented changes being

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8 This process and its outcomes are referred to as data cleaning. Although data cleaning is sometimes regarded with suspicion because it could constitute manipulation to achieve desired research outcomes, when used judiciously it is advocated as being essential to ensure the validity of data. See Hellerstein (2008), “Data cleaning” (n.d.), Osborne and Overbay (2004), and Van den Broeck, Cunningham, Eeckels, and Herbst (2004).
made to only 2.8% of the data values going forward for analysis. Particular care was taken in alterations made to the data of participants from the active treatment groups to avoid substituting values that would work in favour of finding a treatment effect and therefore lend undue support to the research hypothesis. This was notably the case for Participants 20 and 76 at Visit 7 (see Table 5.24).
### Table 5.22

*Adjustments Within Baseline Data to Manage Aberrant Readings*

<table>
<thead>
<tr>
<th>Participant</th>
<th>Group</th>
<th>Foot</th>
<th>Original score</th>
<th>Altered score</th>
<th>Reason for alteration / action</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>C</td>
<td>LbTBI</td>
<td>1.12</td>
<td>.35</td>
<td>This reading was high for that foot on that day only. Substitute it with the average of Visits 4, 6, &amp; 7 for that foot.</td>
</tr>
<tr>
<td>31</td>
<td>P</td>
<td>HbTBI</td>
<td>1.23</td>
<td>.64</td>
<td>Almost all other readings on other visits were around 0.70, so the lowest HbTBI from Visit 4 was used.</td>
</tr>
<tr>
<td>118</td>
<td>L</td>
<td>HbTBI</td>
<td>1.02</td>
<td>.80</td>
<td>This person is problematic. The readings that day seem atypically high. A reading of 0.80 would be suitable after inspecting all the other data points for the HbTBI foot.</td>
</tr>
</tbody>
</table>

* Groups are coded C. Control; H. high GTN; L. low GTN; P. placebo.

### Table 5.23

*Adjustments Within Visit 3 Data to Manage Aberrant Readings*

<table>
<thead>
<tr>
<th>Participant</th>
<th>Group</th>
<th>Foot</th>
<th>Original score</th>
<th>Altered score</th>
<th>Reason for alteration / action</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>L</td>
<td>HbTBI</td>
<td>1.02</td>
<td>.80</td>
<td>This person had very high readings only on HbTBI at Visit 3. The average of the readings at Visit 4 was used instead.</td>
</tr>
<tr>
<td>50</td>
<td>P</td>
<td>LbTBI</td>
<td>1.31</td>
<td>.90</td>
<td>The readings for both feet at Visit 3 seem to be abnormally high. Readings taken from Visit 4 were used instead. However, those figures are just the lowest of readings that were taken after baseline (when one of the LbTBI readings was very low), almost all of which were high. This person had high readings, and large fluctuations between readings.</td>
</tr>
<tr>
<td>55</td>
<td>C</td>
<td>LbTBI</td>
<td>1.12</td>
<td>.70</td>
<td>All readings on this foot (but not the HbTBI foot) were atypically high on this visit. The average of LbTBI readings for Visits 4, 5, 6, &amp; 7 was used instead.</td>
</tr>
<tr>
<td>95</td>
<td>L</td>
<td>LbTBI</td>
<td>1.13</td>
<td>.72</td>
<td>The readings for both LbTBI and HbTBI at this visit seem atypically high for this person. The LbTBI foot was changed to 0.72 and the HbTBI foot was left unchanged at 0.90.</td>
</tr>
<tr>
<td>96</td>
<td>P</td>
<td>HbTBI</td>
<td>1.15</td>
<td>.84</td>
<td>The readings for both feet at this visit seem unusually high given all other readings for this person, except for Visit 5 when both feet were again high. The substituted readings were taken from Visit 4.</td>
</tr>
</tbody>
</table>

* Groups are coded C. Control; H. high GTN; L. low GTN; P. placebo.
Table 5.24

Adjustments Within Visit 7 Data to Manage Aberrant Readings

<table>
<thead>
<tr>
<th>Participant</th>
<th>Group</th>
<th>Foot</th>
<th>Original score</th>
<th>Altered score</th>
<th>Reason for alteration / action</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>P</td>
<td>LbTBI</td>
<td>.09</td>
<td>.33</td>
<td>The original reading was very atypical of other readings for that foot on that day and other days. A more typical reading was chosen. (Winsorising to 0.28 was also possible, but less typical of this particular participant.)</td>
</tr>
<tr>
<td>20</td>
<td>H</td>
<td>LbTBI</td>
<td>.11</td>
<td>.29</td>
<td>This reading was very atypical of the day and other days. The next lowest reading on that foot on that day was chosen, but given the pattern of readings since Visit 3, a more typical reading from this foot, at Visit 7 would have been 0.57. This value was not used, however, in case it favoured the research hypothesis unduly.</td>
</tr>
<tr>
<td>70</td>
<td>L</td>
<td>LbTBI</td>
<td>.15</td>
<td>.22</td>
<td>This person’s LbTBI was always low. It could be Winsorised for the sake of parametric statistics (the next lowest TBI was 0.30), but that would be a distortion of that person’s data, so a midway position of 0.22 was entered, which was typical of readings on the two previous visits.</td>
</tr>
<tr>
<td>50</td>
<td>P</td>
<td>LbTBI</td>
<td>1.08</td>
<td>95</td>
<td>This person’s TBIs fluctuated a lot. The score of 1.08 was Winsorised to help attain a normal distribution of scores.</td>
</tr>
<tr>
<td>76</td>
<td>H</td>
<td>HbTBI</td>
<td>.13</td>
<td>.22</td>
<td>Although .13 is representative of this person’s scores it was a noticeable outlier, so in order to permit parametric statistics it needed to be moved up, and choosing 0.22 seemed to be as low as was reasonable without distorting the normal curve. This was a difficult decision because of fluctuating data throughout. The value of 0.22 seemed to be a slightly more typical reading based on a number of readings. (It could have been Winsorised to .30 even, but that would have distorted the real data and perhaps advantaged the high GTN group so was avoided).</td>
</tr>
<tr>
<td>79</td>
<td>C</td>
<td>LbTBI</td>
<td>85</td>
<td>77</td>
<td>In the control group, the LbTBI of 0.85 was an outstanding outlier in the data. The next data point down from 0.85 was 0.70. The ability to perform parametric stats is jeopardised unless some adjustment is made. This data point could have been Winsorised to 0.71, but this person often had very high TBIs, so the 0.85 was brought down to 0.77. The HbTBI foot reading was reduced from 0.89 to 0.79 to maintain the parallel for that day–even though the HbTBI foot was not an outlier.</td>
</tr>
</tbody>
</table>

* Groups are coded C. Control; H. high GTN; L. low GTN; P. placebo.
5.3.4 Additional and revised information about participants’ TBIs

The next five subsections deal with issues of data management, data cleaning, and additional descriptions of TBIs. They comprise identification of participants with highly fluctuating TBI data, assessment of the extent to which the smaller cuff size influences TBI readings, adjustment of TBI data in light of that assessment, TBI correlations and differences between feet, and stability of TBIs over time.

5.3.4.1 Identification of participants with highly fluctuating TBI data

As part of the above processes of data adjustment, a number of participants with high TBI fluctuations were identified. In order to provide a sense of the inconsistent data obtained from these highly fluctuating participants, the TBI readings taken from three participants are reproduced in Table 5.25. The first set of readings is from a participant who might be regarded as typical of many participants. It is characterised by reasonable consistency between the three measures taken on the same visit and no great fluctuations between visits or between the right and left feet on the same day. The readings from the two inconsistent participants, in contrast, contain some large discrepancies between serial measures on the same occasion and some significant fluctuations between visits and on the contra-lateral sides. The difficulties interpreting, and relying on, such data sets lie in the wide ranges presenting, and in the intermittent juxtaposition of relatively low and high readings.

On close inspection, 14 participants seemed particularly prone to these variations. They could be identified as having produced discrepant readings of $\geq 0.20$ between the three readings on a particular foot on four or more (i.e., at least one third) of the 12 readings made across the six visits at which TBI data were recorded. When the health status information about these participants was checked, likely reasons for high variations in blood pressure were found in each case. Three of these people acknowledged irregularity in taking their prescribed antihypertensive medicine during the study. Four others were on two or three antihypertensive medications, indicating more severe hypertensive disease. Five additional people had respiratory disease. Raynaud’s phenomenon was present in two participants, one of whom was highly unusual in terms of a very early start on antihyperlipidaemic medication at 18 years of age. Another person described a history of painfully cold hands and feet since
childhood, suggesting a congenital disorder of peripheral perfusion, and another participant had atrial fibrillation which could result in unstable blood pressure due to inconsistencies in cardiac output. Table 5.26 contains expanded details concerning these participants from records made both at the start of, and during, the experiment.

Table 5.25

One Consistent and Two Inconsistent Sets of TBI Readings From Participants

<table>
<thead>
<tr>
<th>Visit</th>
<th>Foot</th>
<th>Consistent participant</th>
<th>Inconsistent participants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Reading</td>
<td>Reading</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>R</td>
<td>.52</td>
<td>.52</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>.33</td>
<td>.31</td>
</tr>
<tr>
<td>2</td>
<td>R</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>R</td>
<td>.62</td>
<td>.57</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>.36</td>
<td>.35</td>
</tr>
<tr>
<td>4</td>
<td>R</td>
<td>.65</td>
<td>.73</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>.47</td>
<td>.45</td>
</tr>
<tr>
<td>5</td>
<td>R</td>
<td>.68</td>
<td>.62</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>.41</td>
<td>.43</td>
</tr>
<tr>
<td>6</td>
<td>R</td>
<td>.50</td>
<td>.48</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>.34</td>
<td>.29</td>
</tr>
<tr>
<td>7</td>
<td>R</td>
<td>.71</td>
<td>.67</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>.41</td>
<td>.41</td>
</tr>
</tbody>
</table>

a This participant (Participant 39) from the placebo group was not totally consistent, but had readings that were typical of those not regarded as problematic.

b These participants (Participant 44 from the control group, and Participant 50 from the placebo group) were among 14 participants whose data were subsequently excluded from some analyses.

c R = right foot; L = left foot.
Table 5.26

Extracts From Files of the 14 TBI-Inconsistent Participants Excluded From Some Analyses

<table>
<thead>
<tr>
<th>Partic. No.</th>
<th>Age</th>
<th>Medical status, lifestyle factors, medications of possible relevance, and comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>70</td>
<td>IDDM 40 years, on 3 antihypertensives</td>
</tr>
<tr>
<td>20</td>
<td>58</td>
<td>NIDDM 16 yrs, insulin 14 yrs, 2 antihypertensives, elevated HbA1c, severe painful diabetic neuropathy</td>
</tr>
<tr>
<td>26</td>
<td>65</td>
<td>IDDM, smoking, sometimes doesn’t take antihypertensives</td>
</tr>
<tr>
<td>44</td>
<td>68</td>
<td>Severe COAD, emphysema, asthma, had lung reduction surgery by visit 5</td>
</tr>
<tr>
<td>50</td>
<td>62</td>
<td>Asthma, irregular in antihypertensive medication</td>
</tr>
<tr>
<td>55</td>
<td>70</td>
<td>Has had painfully cold feet and hands since childhood. Severe cramps relieved by carbamazepine</td>
</tr>
<tr>
<td>64</td>
<td>72</td>
<td>Started on calcium channel blocker antihypertensive at Visit 6 to treat Raynaud’s in hands</td>
</tr>
<tr>
<td>67</td>
<td>62</td>
<td>Cardiac arrhythmia, Beta blocker, and major antidepressant</td>
</tr>
<tr>
<td>102</td>
<td>74</td>
<td>Atrial fibrillation, warfarin, Buerger’s sign (100% specific for severe PAD)</td>
</tr>
<tr>
<td>107</td>
<td>73</td>
<td>Diabetes of unknown duration, warfarin, 2 antihypertensives</td>
</tr>
<tr>
<td>110</td>
<td>42</td>
<td>Commenced on hypolipidaemics at 18 yo</td>
</tr>
<tr>
<td>116</td>
<td>64</td>
<td>Asthma, high BMI</td>
</tr>
<tr>
<td>117</td>
<td>65</td>
<td>MI, enlarged heart, emphysema, asthma, hx 30 yr smoking hx, long term hypertension of 45 years duration, morbid obesity, kidney failure, on 3 antihypertensives</td>
</tr>
<tr>
<td>122</td>
<td>38</td>
<td>Raynaud’s hands and feet, severe neurological pain post mastectomy, cold feet since Tamoxifen</td>
</tr>
</tbody>
</table>

Because of the inconsistent data obtained from them, these 14 participants were omitted from some subsequent analyses when it was considered particularly advantageous or necessary to use the more reliable cleaned data rather than data that were representative of the total sample but contaminated by extraneous influences. Whenever the data from these people are omitted from analyses, it is explicitly acknowledged in the text or in a table footnote.

Apart from the 14 participants with noticeably inconsistent TBI readings, 15 additional participants exhibited unusual TBI patterns that were detected either from the computer-based search to identify extreme fluctuations among sets of TBI readings or from the visual scan of the data. Their medical records were consulted after these
participants had been identified, and extracts from those records are provided in Table 5.27. These extracts demonstrate that there were probable medical explanations for the TBI inconsistencies that had been identified. These participants were included in all analyses despite having unusual patterns of TBIs. Their inclusion demonstrates that it was, at times, difficult to obtain consistent data with this cohort of participants.

Table 5.27

Extracts From Files of 15 TBI-Inconsistent Participants Who Were Included in All Analyses

<table>
<thead>
<tr>
<th>Partic. No.</th>
<th>Age</th>
<th>Medical status, lifestyle factors, medications of possible relevance, and comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>80</td>
<td>Diabetes 12 years, emphysema, 2 antihypertensives</td>
</tr>
<tr>
<td>7</td>
<td>70</td>
<td>Severe PAD awaiting surgical intervention, emphysema</td>
</tr>
<tr>
<td>41</td>
<td>83</td>
<td>12 years diabetes, cardiac arrhythmia</td>
</tr>
<tr>
<td>51</td>
<td>88</td>
<td>Severe PAD, hx of DVTs, femoral stenosis, compression stockings, ulcerative colitis, breast cancer, colostomy</td>
</tr>
<tr>
<td>83</td>
<td>81</td>
<td>Diabetes 15 years</td>
</tr>
<tr>
<td>88</td>
<td>70</td>
<td>Kidney transplant recipient, hx of 8.5 years of dialysis, insulin for cortisone induced diabetes</td>
</tr>
<tr>
<td>90</td>
<td>67</td>
<td>Carotid artery stented, very high pain from prolapsed spinal disc, failed surgery, undergoing various interventions for pain mx</td>
</tr>
<tr>
<td>91</td>
<td>68</td>
<td>Very high and fluctuating brachial systolic BPs at times &gt; 200mm Hg, Hx of pulmonary emboli, DVT and collapsed lungs, very stressed by current domestic issues, warm socks associated with ↑ skin temp and TPI increases (?)</td>
</tr>
<tr>
<td>95</td>
<td>64</td>
<td>Psoriatic arthritis, 24 years smoker, methotrexate, major antidepressant</td>
</tr>
<tr>
<td>96</td>
<td>83</td>
<td>Blood clotting abnormality, on warfarin and 2-3 antihypertensives</td>
</tr>
<tr>
<td>97</td>
<td>86</td>
<td>Increase at Visit 7, possibly due to change to ugg boots + warm socks (?)</td>
</tr>
<tr>
<td>104</td>
<td>77</td>
<td>Raynaud’s phenomenon in feet</td>
</tr>
<tr>
<td>111</td>
<td>80</td>
<td>Parkinson’s, emphysema, asthma, bronchitis, smoked for 60 years from 6 yrs old, had 6 clots within 12 months in heart, lungs and leg, 11 years diabetes, cervical fracture with associated nerve damage</td>
</tr>
<tr>
<td>120</td>
<td>68</td>
<td>On urgent wait list for vascular surgery, claudication of 100 m reduced to 20 m during study duration. Ex 70 /day smoker with 35 yr hx of smoking, ex very heavy drinker, high BMI</td>
</tr>
<tr>
<td>123</td>
<td>67</td>
<td>Sleep apnoea, morbid obesity—BMI now 49, was much higher (previously topped 230 kg). Awaiting spinal surgery for chronic back pain, 20 yrs diabetes and hypertension, warfarin, oxycodone HCl, temazepam, digoxin</td>
</tr>
</tbody>
</table>
5.3.4.2 Reanalysis of cuff size influence

In light of the evidence that cuff sizes do indeed influence TBIs to a statistically significant extent (see Section 5.3.2.4 above), it was decided to adjust the data with an amount representing the most consistent difference attributable to cuff size. This was to maximise accuracy of TBIs independent of cuff size. To determine the amount of adjustment as accurately as possible, data from only the TBI-consistent 86 people were used because they were more likely to be valid. Given that there were nearly equal numbers of people with small and large cuffs, it was assumed that on average the two groups would not differ in their real averages. Table 5.28 contains entries concerning the TBIs of participants with both small and large cuffs. They reveal that on average participants with small cuffs had readings that were 0.046 higher on the LbTBI foot and 0.083 higher on the HbTBI foot.

Table 5.28
Differences in TBI Readings for Small and Large Cuffs at Baseline After Data Had Been Cleaned

<table>
<thead>
<tr>
<th>Foot</th>
<th>Small cuff (n = 35)</th>
<th>Large cuff (n = 51)</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>LbTBI</td>
<td>.540</td>
<td>.162</td>
<td>.494</td>
</tr>
<tr>
<td>HbTBI</td>
<td>.653</td>
<td>.168</td>
<td>.570</td>
</tr>
</tbody>
</table>

* Data were analysed separately for each foot from the 86 TBI-consistent participants.

In light of these differences, 0.06 was subtracted from each TBI from small cuff data. The figure of 0.06 was chosen because it was the integer closest to the average of the two mean differences. This was in order to permit economy of description in this research as well as to act as a readily applicable clinical recommendation to overcome the differences intrinsic in the different cuff size measurements.

As a result of the above adjustment, there were no longer any differences in the total sample (N = 100) between the average TBIs for those with whom small versus large cuffs had been used. For the LbTBI foot \( t(98) = 1.30, p = 0.198 \) (means of 0.458 and 0.500 for small and large cuffs respectively), and for the HbTBI foot \( t(98) = 0.37, p = \)
0.714 (means of 0.596 and 0.584 for small and large cuffs respectively). Furthermore, Levene tests indicated that there was no difference in variability associated with cuff size on either of the two feet. For the LbTBI foot, $F = 0.48, p = 0.489$, and for the HbTBI foot, $F = 0.02, p = 0.879$.

5.3.4.3 Final TBI data profile for analysis

Inspection of the data following adjustment for cuff size revealed only one outlier, an extremely low value of 0.10 on the LbTBI foot at Visit 3 for a participant in the control group. Because the next TBI in the control group’s distribution was 0.33, this TBI was increased to 0.25, which was more typical of that person over a number of readings. This was the final data point altered. In total, therefore, 18 TBIs for 11 people were altered—amounting to only, and exactly, 3% of the data. Six of the 18 altered data points occurred for the 14 participants in the TBI-inconsistent group, so in the analyses from which those people’s data were excluded, only 2% of the data had been adjusted.

At this point it was finally possible to describe the sample accurately in terms of TBIs and to determine whether the group allocation process had resulted in an even spread of baseline TBIs across the four groups. Table 5.29 contains the relevant information. The upper row of each pair of rows in that table refers to the full sample of 100 participants. It is evident that there are only slight deviations in each group from the mean. On the LbTBI foot, the high GTN and control groups are only 0.036 and 0.005 below the total mean respectively, and the low GTN and placebo groups are both only 0.016 greater than the total mean, demonstrating their proximity and near equality in absolute differences. On the HbTBI foot, both low GTN dose and placebo groups are 0.018 below the total mean, and the high GTN dose and control groups, 0.028 and 0.018 above the total mean respectively.

The lower row of each pair of rows in Table 5.29 contains entries relevant to the 86 TBI-consistent participants. Within these 86 people, the subgroups exhibit average TBI differences that for both feet are similarly small to those identified across the subgroups based on the total sample. When compared with the full sample, these 86 people have

---

9 Because this person was in the control group, adjusting his score upward not only eliminated it as an outlier but also avoided any GTN treatment effect between baseline and Visit 3 being spuriously inflated in subsequent analyses.
slightly higher LbTBIs in all subgroups except the placebo group, where the reverse occurs. In contrast, for the HbTBI foot, relative to the full sample, the 86 people have slightly lower TBIs, except for the high GTN group, which is slightly higher.

Table 5.29

TBIs at Baseline for Total Sample and the Subsample of 86 TBI-Consistent Participants Based on the Lowest TBI Readings Following Final Data Adjustment

<table>
<thead>
<tr>
<th>Statistic</th>
<th>Total sample (N = 100, 86)</th>
<th>Low GTN dose (n = 33, 29)</th>
<th>High GTN dose (n = 24, 22)</th>
<th>Placebo (n = 22, 19)</th>
<th>Control (n = 21, 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foot</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LbTBI</td>
<td>.482 (.160)</td>
<td>.466 (.162)</td>
<td>.518 (.149)</td>
<td>.466 (.161)</td>
<td>.487 (.167)</td>
</tr>
<tr>
<td></td>
<td>86</td>
<td>.488 (.159)</td>
<td>.475 (.159)</td>
<td>.528 (.147)</td>
<td>.458 (.152)</td>
</tr>
<tr>
<td>HbTBI</td>
<td>.598 (.166)</td>
<td>.571 (.154)</td>
<td>.617 (.137)</td>
<td>.571 (.175)</td>
<td>.607 (.201)</td>
</tr>
<tr>
<td></td>
<td>86</td>
<td>.579 (.163)</td>
<td>.558 (.152)</td>
<td>.620 (.143)</td>
<td>.560 (.167)</td>
</tr>
</tbody>
</table>

Min.– max.

<table>
<thead>
<tr>
<th>Statistic</th>
<th>Total sample (N = 100, 86)</th>
<th>Low GTN dose (n = 33, 29)</th>
<th>High GTN dose (n = 24, 22)</th>
<th>Placebo (n = 22, 19)</th>
<th>Control (n = 21, 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foot</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LbTBI</td>
<td>.13–.76</td>
<td>.13–.70</td>
<td>.23–.76</td>
<td>.23–.75</td>
<td>.27–.76</td>
</tr>
<tr>
<td></td>
<td>86</td>
<td>.18–.76</td>
<td>.18–.70</td>
<td>.23–.76</td>
<td>.23–.75</td>
</tr>
<tr>
<td>HbTBI</td>
<td>.19–.91</td>
<td>.24–.85</td>
<td>.32–.83</td>
<td>.27–.82</td>
<td>.19–.91</td>
</tr>
<tr>
<td></td>
<td>86</td>
<td>.19–.86</td>
<td>.24–.85</td>
<td>.32–.83</td>
<td>.27–.81</td>
</tr>
</tbody>
</table>

*a Based on total sample (upper row of each pair) and subsample of 86 participants with reliable TBIs (lower row of each pair) after adjusting data from participants wearing small cuffs.

The standard deviations, and minimum and maximum values, are similar both across all subgroups from the total sample and across the subgroups comprising the 86 TBI-consistent participants. This again demonstrates similarities in TBIs, confirming the conclusion that the distributions of baseline TBIs was similar across all four groups for both feet.
A comparison of the TBIs of the four groups prior to, and following, the adjustments to the data reveal some important differences in their relationship to each other. These differences are evident by comparing entries in Table 5.13 (Section 5.3.2.1.1) with those in Table 5.29. The high GTN and control groups interchanged positions on the LbTBI foot: The control group originally had the highest average TBIs but became second-highest because its TBIs dropped by 0.12 points. The high GTN dose group, despite dropping by 0.07 points, became the group with the highest TBIs. On the HbTBI feet, the low GTN group, which was originally had the highest average TBIs, became equal lowest, matching the placebo group. These changes reveal that the data management processes altered both the order and magnitude of the baseline data across the four groups in ways that could affect the substantive analyses of this research and raise the possibility that leaving the data in their original form could have produced somewhat different, and possibly misleading, results.

Table 5.30 contains descriptive statistics for the LbTBI and HbTBI feet at baseline, Visit 3, and Visit 7 for all 100 participants. (Baseline entries in that table duplicate some entries in Table 5.29.) Entries in Table 5.30 indicate that on the LbTBI feet, average TBIs in the low GTN dose group increased by a moderate 0.06 between baseline and Visit 3, then continued with a further slight increase of 0.01 by Visit 7. The high GTN dose group’s average TBIs increased by a greater 0.11 between baseline and Visit 3, but by Visit 7 had lost most of that increase, finishing only 0.04 above baseline. The placebo group matched the high GTN dose group with an initial average increase of 0.11, and then remained steady at the final visit. The control group initially increased by an average of 0.50, then dropped by 0.70 to below its baseline level. These unrefined comparisons are analysed in greater depth, and with more sophistication, using ANCOVAs in Section 5.5.8 below.

On the HbTBI feet (which had not been exposed to GTN treatment in any group), the changes were much smaller overall, with the maximum average increase of 0.05 evident in the placebo group at Visit 3, followed by a 0.03 decrease at Visit 7. All other groups had only small average changes, with none greater than 0.03. These comparisons are included in the ANCOVAs reported in Section 5.5.8.
### Table 5.30

**Final TBIs of LbTBI and HbTBI Feet at Baseline, Visit 3, and Visit 7 for the Four Subgroups**

<table>
<thead>
<tr>
<th>Statistic</th>
<th>Foot</th>
<th>Time</th>
<th>Low GTN dose ((n = 33))</th>
<th>High GTN dose ((n = 24))</th>
<th>Placebo ((n = 22))</th>
<th>Control ((n = 21))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LbTBI</td>
<td>Baseline</td>
<td>.466 (.162)</td>
<td>.518 (.149)</td>
<td>.466 (.161)</td>
<td>.487 (.167)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Visit 3</td>
<td>.529 (.193)</td>
<td>.621 (.220)</td>
<td>.582 (.183)</td>
<td>.543 (.172)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Visit 7</td>
<td>.538 (.192)</td>
<td>.540 (.195)</td>
<td>.576 (.189)</td>
<td>.470 (.178)</td>
<td></td>
</tr>
<tr>
<td>HbTBI</td>
<td>Baseline</td>
<td>.571 (.154)</td>
<td>.617 (.137)</td>
<td>.571 (.175)</td>
<td>.607 (.201)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Visit 3</td>
<td>.564 (.202)</td>
<td>.600 (.206)</td>
<td>.620 (.165)</td>
<td>.591 (.189)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Visit 7</td>
<td>.581 (.210)</td>
<td>.589 (.190)</td>
<td>.599 (.229)</td>
<td>.577 (.187)</td>
<td></td>
</tr>
</tbody>
</table>

Min.– max.

| LbTBI          | Baseline| .13–.70 | .23–.76 | .23–.75 | .27–.76 |
|                | Visit 3| .18–.85 | .21–.85 | .30–.86 | .25–.89 |
|                | Visit 7| .27–.90 | .17–.82 | .28–.89 | .15–.77 |
| HbTBI          | Baseline| .24–.85 | .32–.83 | .27–.82 | .19–.91 |
|                | Visit 3| .18–.91 | .15–.92 | .27–.86 | .12–.89 |
|                | Visit 7| .14–1.00 | .22–1.00 | .20–1.00 | .27–.94 |

\(^a\) Based on total sample after adjusting data from participants wearing small cuffs and from one additional person in the control group with an outlying score of .04 on the LbTBI foot at Visit 3.

The histograms in Figure 5.5, Figure 5.6, and Figure 5.7 refer to the distributions of scores at baseline, Visit 3, and Visit 7 respectively. Some departures from normal distributions can be seen in those histograms, particularly in the slightly bimodal pattern at baseline on the LbTBI foot.
Figure 5.5. Histograms showing distributions of lowest TBI readings from LbTBI and HbTBI feet at baseline after adjustments for cuff sizes. $N = 100$.

Figure 5.6. Histograms showing distributions of lowest TBI readings from LbTBI and HbTBI feet at Visit 3 after adjustments for cuff sizes. $N = 100$.

Figure 5.7. Histograms showing distributions of lowest TBI readings from LbTBI and HbTBI feet at Visit 7 after adjustments for cuff sizes. $N = 100$. 
Despite the lack of normality, skewness and kurtosis for all six distributions were within the conventionally accepted bounds of ±1, with the exceptions that the LbTBI foot had kurtosis of -1.04 and -1.10 at baseline and Visit 7 respectively. Kolmogorov-Smirnov tests indicated that both of the associated distributions departed from normality, $p < .009$ and $p < .045$ respectively.

**Information about analyses in Sections 5.3.4.4 to 5.3.6.3**

At this point, with valid TBI data it was possible to describe some additional features about TBIs more accurately than would have previously been possible. It was also possible to explore how TBIs were related to both demographic and health status variables to provide a more in-depth and informative profile of the participants in this study. This information could also assist to identify ways in which subsequent analyses might be both more effective (for example by indicating appropriate covariates for ANCOVAs) and more specifically focused (for example by exploring relationships between TBIs and targeted demographic or health status variables), as well as enhance the interpretation of subsequent results.

To these purposes, Sections 5.3.4.4 and 5.3.4.5 immediately below are concerned with the relationships of TBIs between the participants’ two feet and the temporal stability of TBIs. Following that, Section 5.3.5 contains five subsections that deal with relationships between TBIs and demographic variables in the sample. Section 5.3.6 contains three subsections that deal with relationships between TBIs and the participants’ health status.

The analyses for all of these subsections, i.e., throughout the remainder of this section (Section 5.3.4) and in Sections 5.3.5 and 5.3.6 were performed initially with nonparametric statistics for three reasons: First, some of the TBI distributions departed slightly from the normal curve; second, in some of the analyses at least one of the variables (e.g., age) was not normally distributed; and third, in some of the analyses the numbers in the groups being compared were extremely different from each other (e.g., when comparing the six current smokers with the 94 participants who were not currently smoking). For many of the analyses, parallel parametric tests were also used and in no instance did discrepancies occur with the results from the nonparametric
analyses, so for the most part only the results from nonparametric tests are reported in order to avoid over-reporting of results and to maintain consistency. However, the results from parametric outcomes are provided when they would enhance interpretation of findings, for example with the provision of mean TBI values. All analyses were initially conducted with data from the full sample ($N = 100$), but if the results seemed to contradict expectations, or to approach but not attain statistical significance, analyses were conducted with data from the smaller sample of 86 TBI-consistent people in case their data were more valid.

5.3.4.4 Correlations and differences between feet

Correlations and differences between the participants’ two feet were analysed in three ways, the first two identifying correlations (associations), and the third identifying whether there was a significant difference between the TBIs of the two feet. The Spearman’s rank-order correlation between the LbTBI and HbTBI feet at baseline was significant, $r_s(98) = .71, p < .001$, indicating that if the TBI of one foot was relatively high, the TBI of the other foot also tended to be relatively high, and vice versa. The corresponding ICC was noticeably lower at .57, indicating that correspondence between the two feet was not as great as the correlation coefficient suggests. This became clearer when a Wilcoxon matched-pairs signed-rank test was used to determine whether the two feet had similar TBIs. It yielded a significant difference, with mean ranks of 32.4 for the LbTBI foot and 52.0 for the HbTBI foot at baseline, $z = 6.8, p < .001$. The respective means were 0.482 and 0.589 (as seen in Table 5.29). Again, therefore, it is evident that the two feet do differ from each other at baseline, with the LbTBI feet having lower TBIs. Furthermore, as before, it is obvious that the difference is statistically significant.

Together, the Spearman’s correlation coefficient, ICC, and Wilcoxon test results demonstrate that the TBI values for both feet tend to correspond with each other when compared with the TBI values across different people, but for most people in this sample one foot had a significantly higher TBI than did the other foot.
5.3.4.5 Stability of TBIs over time

Analyses of stability of TBIs over time were conducted solely with data from the control group because of the prospect of contamination from treatment/placebo effects in the other groups on post-baseline measures.

Spearman’s rank order correlations for each foot between baseline and Visit 3 indicate a reasonably high degree of consistency across the 2 months. For the LbTBI foot, \( r_s(19) = .74, p < .001 \), and for the HbTBI foot, \( r_s(19) = .67, p = .021 \). The strength of the relationship was maintained over the 6-month period from baseline to Visit 7. For the LbTBI foot, \( r_s(19) = .77, p < .001 \), and for the HbTBI foot, \( r_s(19) = .68, p < .001 \).

ICCs for the LbTBI foot and the HbTBI foot were .68 and .66 respectively from baseline to Visit 3, and .77 and .64 for the two respective feet from baseline to Visit 7. In combination with the moderately strong correlation coefficients, the ICCs suggest that even though they remained relatively stable when juxtaposed with other people’s TBIs, many individuals’ toe pressures did change to some extent across the 2- and 6-month time spans.

Again using data from only the control group, the Wilcoxon matched-pairs signed-ranks test was used to determine first whether the TBIs in each foot moved significantly up or down from baseline to Visit 3. TBIs in the LbTBI foot, with ranks of 9.3 (baseline) and 12.1 (Visit 3) yielded a \( z \) value of 1.44, \( p = .149 \), indicating that they did not move systematically higher or lower from one time to the other across the 2-month time period. On the HbTBI foot, from baseline to Visit 3, the Wilcoxon test, with ranks of 9.7 (baseline) and 10.3 (Visit 3) yielded a \( z \) value of 0.32, \( p = .747 \). Again, this was not significant. Across the 2-month period, therefore, the readings did not move systematically up or down for the control group, as might be expected.

A Wilcoxon test was also used to assess whether differences occurred across the 6-month period between baseline and Visit 7. TBIs on the LbTBI foot, with ranks of 10.7 (baseline) and 9.3 (Visit 7) yielded a \( z \) value of 0.46, \( p = .643 \), indicating that they did not move systematically higher or lower from one time to the other. On the HbTBI foot,
with ranks of 11.9 (baseline) and 7.9 (Visit 7) the Wilcoxon test yielded a $z$ value of 0.97, $p = .334$. Again, this was not significant. Across the 6-month period, therefore, the TBIs did not move systematically up or down.

As another means of assessing the consistency of TBIs across time, absolute differences from baseline to Visit 3, and from baseline to Visit 7, again with the control group only, provide some additional insights. These are shown in Table 5.31. They indicate that, on average, people in the control group moved either higher or lower by approximately 0.100 (range from 0.094 to 0.130) from baseline to both of the other time periods. Neither foot seemed to be more likely to change than did the other.

Analysis of percentiles regarding these absolute differences indicates that the TBIs on one or both feet moved by approximately 0.100 for 50% of the sample between baseline and Visit 3, and to the same extent, again for 50% of the sample, between baseline and Visit 7. The TBIs differed on one or both feet by more than 0.200 from baseline to Visit 3 for 25% of the sample, and by the same amount from baseline to Visit 7 on the HbTBI foot for 25% of the sample. However, across the baseline to Visit 7 period, the LbTBI foot’s TBIs moved to a smaller extent: only 0.130 or more for 25% of the sample. As can be seen from the final column of Table 5.31, taking both feet into account across both the 2- and 6- month periods, the maximum change was .38.

Table 5.31

**Absolute Differences Between TBI Readings From Baseline to Visit 3 and Baseline to Visit 7**

<table>
<thead>
<tr>
<th>Timepoints</th>
<th>Foot</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline to Visit 3</td>
<td>LbTBI</td>
<td>.121</td>
<td>.088</td>
<td>.01</td>
<td>.27</td>
</tr>
<tr>
<td></td>
<td>HbTBI</td>
<td>.130</td>
<td>.101</td>
<td>.00</td>
<td>.38</td>
</tr>
<tr>
<td>Baseline to Visit 7</td>
<td>LbTBI</td>
<td>.094</td>
<td>.077</td>
<td>.00</td>
<td>.31</td>
</tr>
<tr>
<td></td>
<td>HbTBI</td>
<td>.123</td>
<td>.116</td>
<td>.00</td>
<td>.36</td>
</tr>
</tbody>
</table>

*a These analyses are based on data from the control group only ($n = 21$).
From baseline to Visit 3, seven participants in the control group exhibited a shift in which the foot with the lower TBI became the higher TBI foot. Two of these were “trivial”—i.e., the TBIs changed by only a couple of points. At Visit 7, two of these remained higher, and the other five reverted to their original lower status. By Visit 7, two more reversals had occurred in that the lower and higher TBIs had exchanged relative position since baseline.

Together, these results indicate that TBIs fluctuated for most control group participants within small to moderate ranges over both 2- and 6-month time periods. Although these changes were analysed as not being statistically significant according to several tests assessing correlations and differences, for nine of the 21 participants (43%), a reversal of the higher and lower TBI rank occurred at some point, and a small number of participants’ TBIs changed to a noticeable extent. Because the TBIs exhibit a degree of temporal instability, particularly for some participants, any treatment effect would need to be sufficiently strong to be distinguishable from normally occurring fluctuations.

5.3.5 Associations of demographic variables with TBIs

In order to identify associations between demographic variables and TBIs, baseline data were used because information could therefore be obtained for the whole sample prior to any treatment/placebo effects.

5.3.5.1 Age and TBIs

Spearman’s rank order correlations between age and TBIs yielded negative but insignificant correlations on both the LbTBI foot, \( r_s(98) = -.16, p = .103 \) as well as on the HbTBI foot, \( r_s(84) = -.15, p = .138 \).

5.3.5.2 Smoking and TBIs

Spearman’s correlations between years of smoking and TBIs on both feet, with only the 44 people who were either ex-smokers \( (n = 38) \) or current smokers \( (n = 6) \), were insignificant. For the LbTBI foot, \( r_s(42) = -.11, p = .453 \), and for the HbTBI foot, \( r_s(42) = .18, p = .233 \). For this group of people, therefore, there is not a relationship between the number of years of smoking and TBIs.
Current smokers, however, did have lower TBIs at baseline: When the sample was divided into the six people (6%) who were currently smoking versus the 94 people (94%) who had either never smoked or had discontinued (regardless of how long they had been smoking—even up to 56 years—prior to discontinuing), for the LbTBI foot a Mann-Whitney $U$ test yielded ranks of 25.6 and 52.1 respectively, $z = 2.17, p = .030$. (The TBI means were 0.347 and 0.491 respectively.) The results were similar for the HbTBI foot, where the ranks were 24.8 and 52.1 for the current smokers versus non-current smokers (including those who had never smoked) respectively, $z = 2.43, p = .025$. (The respective means were 0.457 and 0.597.)

Inspection of the data revealed that the six participants who currently smoked were long-term smokers: Five of them had smoked for more than 45 years, and the remaining person had smoked for 20 years.

### 5.3.5.3 Alcohol consumption and TBIs

A Mann-Whitney $U$ test comparing the people who were drinking an excessive amount of alcohol ($n = 11$; 7 men, 4 women) with everyone else ($n = 89$) indicated no difference between the two groups for either the LbTBI foot, $z = 1.10, p = .266$ or for the HbTBI foot, $z = 0.03, p = .978$. A Spearman’s rank order correlation between the amount of alcohol consumed and TBIs across the total sample also indicated no relationship between those two variables. For the LbTBI foot, $r_s(98) = –.02, p = .840$ and for the HbTBI foot, $r_s(98) = –.04, p = .690$.

### 5.3.5.4 BMI and TBIs

A Spearman’s rank correlation between BMIs and TBIs, across the total sample indicated no relationship between those two variables. For the LbTBI foot, $r_s(98) = .15, p = .132$ and for the HbTBI foot, $r_s(98) = .14, p = .155$.

### 5.3.5.5 Mobility and TBIs

A Mann-Whitney $U$ test comparing the people who had impaired mobility ($n = 17$) with those who did not ($n = 83$) indicated no difference between the two groups for either the LbTBI foot, $z = 0.50, p = .960$ or for the HbTBI foot, $z = 1.21, p = 226$. 
5.3.6 Association of health status with TBIs

As with the associations between demographic variables and TBIs, baseline data were used for identifying associations between medical condition variables and TBIs because that information could be obtained for the whole sample prior to any treatment/placebo effects.

5.3.6.1 Diabetes and TBIs

Three sets of analyses were used to determine the relationship of TBIs with diabetes. First, people who had diabetes (either type 1 or type 2, n = 58) were compared with those who did not (n = 42). A Mann-Whitney U test indicated no difference between these groups for either the LbTBI foot, \( z = 0.03, p = .975 \) or the HbTBI foot, \( z = .0.45, p = .652 \).

Second, of those who had diabetes, people who had been diagnosed with it for less than 10 years (n = 17) were compared with those who had been diagnosed with diabetes for 10 or more years (n = 41). A Mann-Whitney U test indicated no difference between these two groups for the LbTBI foot, \( z = 1.41, p = .159 \), but there was a significant difference between them for the HbTBI foot, \( z = 2.29, p = .022 \), where the two groups’ ranks were 21.6 and 32.8 respectively (TBI means of 0.503 and 0.612 respectively).

Third, of those who had diabetes, people who were not taking insulin medication were compared with those who were. A Mann-Whitney U test comparing these two groups indicated no difference between them for either the LbTBI foot, \( z = 0.34, p = .737 \) or the HbTBI foot, \( z = 0.81, p = .416 \).

There was also no correlation between HbA1c and TBIs. For the LbTBI foot, \( r_s(50) = -.12, p = .392 \) and for the HbTBI foot, \( r_s(50) = -.12, p = .407 \).

Overall, these results indicate a lack of relationship between TBIs and either diabetes or diabetes control in this sample.
5.3.6.2 Antihypertensive medication and TBIs

Results of the analyses concerning people on antihypertensive medication and TBIs were contradictory. For the LbTBI foot, when comparing the TBIs of people who were taking antihypertensive medication \((n = 79)\) with those not taking that medication \((n = 21)\), a Mann-Whitney \(U\) test yielded ranks of 47.9 and 60.3 respectively. This difference in ranks was not significant, \(z = 1.74, p = .082\). Because this finding seemed counterintuitive, a subsequent analysis was performed with data from only the 86 people whose TBI readings were consistent. For the LbTBI foot, The Mann-Whitney \(U\) test yielded ranks of 40.2 for the 68 people on antihypertensive medication and 55.9 for the 18 people not on medication respectively, \(z = 2.38, p = .017\). (Mean TBIs were 0.468 and 0.565 respectively.)

For the HbTBI feet, the Mann-Whitney \(U\) tests yielded insignificant results for both the 100 and 86 people samples. For the 100 people, the ranks were 49.5 and 54.2 respectively, \(z = 0.66, p = .51\). For the 86 people, the ranks were 42.2 and 48.3 respectively, \(z = 0.93, p = .353\).

Taken together, these results indicate a lack of relationship between TBIs and use of hypertensive medication. However, the significantly higher TBIs on the LbTBI feet of people taking hypertensive medication should not be overlooked given that this finding was based on data that were more trustworthy.

5.3.6.3 Hypolipidaemic medication and TBIs

Mann-Whitney \(U\) tests indicated that there were no differences in TBIs for people taking hypolipidaemics \((n = 65)\) versus those not taking them \((n = 35)\). For the LbTBI foot, ranks were 48.8 and 55.2 respectively, \(z = 1.33, p = .184\). For the HbTBI foot, ranks were 47.2 and 55.2 respectively, \(z = 0.56, p = .578\).

5.3.7 Possible side effects and comparison of contralateral foot responses

Fourteen of the 57 participants in the two treatment groups reported what could be regarded as side effects during the trial. This represents 25% of those exposed to the intervention. Seven of these participants were in the low GTN dose group, representing 21% of that group, and the other seven were in the high GTN dose group, representing
29% of that group. Among these 14 people, 12 (six in each group) experienced headaches, the most common side effect of GTN exposure; two (one in each group) had a localised rash at the patch application site; and one man (in the low GTN group) attributed the return of his previously lost erectile function post prostate surgery to the intervention.

The headaches always occurred within the first day of GTN exposure, and the majority were of 2 days duration. They were invariably rated as mild or very mild, and only two of the 12 participants who experienced headaches used any pain relief medication to alleviate the pain.

A single episode of headache and dizziness for one participant (Participant 47 in the high GTN group) did not occur until after Visit 3, and this could not be attributed to GTN as it occurred after temporarily ceasing the patch treatment. For another participant (Participant 81 in the high GTN group), the headache was later associated with low blood glucose levels (BGLs), so again could be attributed to something other than GTN.

Both participants with skin reactions had this effect develop only after 3 months of exposure to the intervention. The patch treatment was able to be continued in each case with adherence to strict rotation of the application without site overlap until the end of the 6-month trial period.

Data about possible side effects and TBIs in both feet were combined to explore the relationship between them and the consequent implications if a relationship did exist. In particular, if headaches were associated with bilateral toe vasodilation a generalised vasodilatory effect might be occurring throughout the body. The relevant information is condensed in two tables: Table 5.32 contains information for only the low GTN dose participants, and Table 5.33 contains information relevant to the high GTN dose participants. As indicated in these tables’ footnotes, the text in these tables is in regular font, bold font, and italics to reflect researcher notes made about participants, results consistent with a generalised effect, and results contrary to a generalised effect respectively.
Table 5.32

Side Effects for Seven Participants in Low GTN Dose Group\(^a\)

<table>
<thead>
<tr>
<th>Pt No.</th>
<th>Side effect</th>
<th>Noted at visit(s)</th>
<th>Onset / Duration(^b)</th>
<th>Comments, particularly relevant to a general vs local effect of GTN</th>
<th>Severity/medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>Headache</td>
<td>3</td>
<td>2 days</td>
<td>By Visit 3, LbTBI foot increased by only 0.12, and the HbTBI foot decreased by 0.02.</td>
<td>Mild/nil</td>
</tr>
<tr>
<td>64</td>
<td>Headache</td>
<td>3-7</td>
<td>Ongoing</td>
<td>At Visit 3, the LbTBI foot increased by 0.41, but the HbTBI foot decreased by 0.28. By Visit 7, the LbTBI foot was up by 0.27, and the HbTBI foot down by 0.13.</td>
<td>Mild/nil</td>
</tr>
<tr>
<td>95</td>
<td>Headache</td>
<td>3</td>
<td>6 days</td>
<td>LbTBI foot increased markedly at Visit 3 as did the HbTBI foot.</td>
<td>Mild/nil</td>
</tr>
<tr>
<td>99</td>
<td>Headache</td>
<td>3</td>
<td>1 day</td>
<td>Not a “GTN responder” in that his LbTBI foot increased by only 0.02 from baseline to Visit 3. The HbTBI foot dropped by 0.17</td>
<td>Mild/nil</td>
</tr>
<tr>
<td>104</td>
<td>Headache</td>
<td>3</td>
<td>2 days</td>
<td>The LbTBI foot increased by 0.20; the HbTBI foot increased by 0.10.</td>
<td>Mild/nil</td>
</tr>
<tr>
<td>110</td>
<td>Headache</td>
<td>3</td>
<td>2-3 weeks</td>
<td>Relieved by Ibuprofen. Slight skin reaction initially. Was able to continue with strict application site rotation. Needed to cease by Visit 7 due to skin rash. The LbTBI foot could not be measured at Visit 3 or at Visit 7. The HbTBI foot’s TBIs decreased progressively from 0.52 at baseline to 0.28 by Visit 3, then to 0.11 by Visit 7.</td>
<td>Mild/Yes</td>
</tr>
<tr>
<td>111</td>
<td>Rash on LbTBI foot</td>
<td>5-7</td>
<td>3 months</td>
<td>After years of no erectile function post prostate surgery, regular erectile effects were reported along with some marked improvement in pedal vibration sense. The LbTBI foot decreased by 0.20 at Visit 3, and this person was not a high responder at Visit 7. His LbTBI foot decreased by 0.09.</td>
<td>N/A</td>
</tr>
</tbody>
</table>

\(^a\) Text in regular font is taken from participant notes; text in bold refers to situations that were consistent with a general effect occurring, but do not prove it; text in italics indicates that a general effect was not occurring.

\(^b\) Onset was associated with commencement of GTN unless expressly stated.

Across both tables, only three of the 12 participants with headaches had concomitant TBI values on the treated and nontreated feet that were consistent with a generalised vasodilatory effect occurring by the time of the follow up measurement four weeks after commencement of GTN treatment. These results do not, however, prove that a generalised effect was occurring; they merely conform to the pattern of TBIs that would occur if a generalised GTN effect was present.
Overall, the italicised entries in both Table 5.32 and Table 5.33 indicate that any vasodilatory effect from GTN had occurred on the treated side alone in the majority of these 14 participants.

Table 5.33

Side Effects for Seven Participants in High GTN Dose Group

<table>
<thead>
<tr>
<th>Pt No.</th>
<th>Side effect</th>
<th>Noted at visit(s)</th>
<th>Onset / Duration</th>
<th>Comments, particularly relevant to a general vs local effect of GTN</th>
<th>Severity/medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Headache</td>
<td>3,4</td>
<td>2 months</td>
<td>“Thick head”. The LbTBI foot increased by 0.28 at Visit 3; the HbTBI foot increased by 0.23.</td>
<td>Mild/nil</td>
</tr>
<tr>
<td>7</td>
<td>Headache</td>
<td>3</td>
<td>2 days</td>
<td>Relieved by Paracetamol. The LbTBI foot did not increase by much if at all by Visit 3 (0.08), and this person’s TBI on the HbTBI foot dropped.</td>
<td>Mild/yes</td>
</tr>
<tr>
<td>41</td>
<td>Headache</td>
<td>3,4</td>
<td>2 days</td>
<td>The LbTBI foot did not increase by much if at all (0.07) and the HbTBI decreased by 0.02</td>
<td>Mild/nil</td>
</tr>
<tr>
<td>47</td>
<td>Headache</td>
<td>4</td>
<td>2 days</td>
<td>Persistent headache and dizziness 24 hours after ceasing patch-(thus not likely to be associated with GTN). Furthermore, the LbTBI foot increased by 0.51, but the HbTBI foot by only 0.11.</td>
<td>Severe 8/10 headache 7/10 dizziness/not stated</td>
</tr>
<tr>
<td>59</td>
<td>Headache</td>
<td>3,4</td>
<td>2 months</td>
<td>Only when sitting still for long periods in the mornings. (Note very low heart rate of 44 BPM). The LbTBI foot showed no notable increases.</td>
<td>Very mild</td>
</tr>
<tr>
<td>81</td>
<td>Headache</td>
<td>3-7</td>
<td>Initially 2 weeks</td>
<td>Later, associated headaches with low BGLs. Relieved by Paracetamol. Furthermore, the LbTBI foot decreased by 0.19 at Visit 3.</td>
<td>Mild/yes</td>
</tr>
<tr>
<td>123</td>
<td>Skin reaction on LbTBI foot</td>
<td>5,6,7</td>
<td>3 months</td>
<td>Was able to persist with patches until end of study with strict site rotation. The LbTBI foot did not increase notably at either Visit 3 or Visit 7.</td>
<td>Mild</td>
</tr>
</tbody>
</table>

---

* Text in regular font is taken from participant notes; text in bold refers to situations that are consistent with a general effect occurring, but do not prove it; text in italics indicates that a general effect was not occurring.

* Onset was associated with commencement of GTN unless expressly stated.

Additional analyses were conducted initially with data from only the 28 participants in the two treatment groups (combined) whose TBIs on the LbTBI foot had increased by > 0.100 from baseline to Visit 3. These people were chosen both because their LbTBI foot had exhibited the anticipated TBI increase resulting from application of the
GTN patch and because the TBI increase was sufficiently large that it was not likely to be the naturally occurring temporal instability that had been identified in Section 5.3.4.5 above.

Across the baseline to Visit 3 timespan (one month of GTN exposure), the pattern of TBI results for only 11% of these participants (3 of the 28 with > 0.100 change in TBI) was consistent with—but did not prove—there being a generalised GTN effect to the other foot. The increases in TBIs for their treated and nontreated feet were similar for these participants. An additional 33% of the results were consistent with there being a generalised but subdued effect to the other foot, for example because the TBI of the nontreated foot increased but to a much lesser extent than did the TBI of the treated foot (with increases such as 0.02 and 0.25 respectively). Once more, however, the pattern of these results did not prove the existence of a generalised effect. The remaining 57% of the results indicated clearly that there was not a generalised effect occurring. This was demonstrated, for example, by the nontreated foot increasing to a much greater extent than did the treated foot (with TBI increases such as 0.27 and 0.17 respectively), or by the nontreated foot decreasing noticeably while the treated foot increased noticeably (with TBI differences between baseline and Visit 3 such as –0.28 and 0.48 respectively).

Similar results were obtained when conducting a parallel comparison involving the 23 participants from the two treatment groups whose TBIs on the LbTBI foot had increased by > 0.100 from baseline to Visit 7. In these analyses, 65% of the participants’ TBI patterns provided clear evidence that a generalised effect was not occurring from one foot to the other, with the residual 35% being consistent with the possibility of, but not proving, the presence of a generalised effect. Of that 35%, only half (17%) of the TBI values were more than moderately consistent with the pattern that would be found if a generalised effect was present.

In combination with the results from Tables 5.32 and 33, these results indicate quite strongly that any influence of GTN was unlikely to have transferred from the treated to the nontreated foot for the majority, and possibly all, of the participants at the time points studied.
5.3.8 TBIs and GTN: ANCOVAs

Although relatively brief, this section (Section 5.3.8) focuses on the primary question of this research: whether application of transdermal GTN can produce increases in TBIs for people who have PAD. To address this question appropriately, a number of ANCOVAs were conducted\(^\text{10}\) and they, and their outcomes, are described in this section. The first set of these ANCOVAs addresses the changes in TBIs from baseline to Visit 3. The subsequent ANCOVAs explore TBI changes from baseline to Visit 7. The validity of these ANCOVAs, and the opportunities and constraints associated with conducting them, rest to a large extent on the results obtained in the analyses that preceded them in the previous 38 pages of this thesis (Results Sections 5.3.2 to 5.3.7).

5.3.8.1 Effect of GTN from baseline to Visit 3

Table 5.34 contains descriptive statistics relevant to the differences in TBIs from baseline to Visit 3. According to these entries, which are based on entries in Table 5.30 in Section 5.3.4.3, on the LbTBI foot there was an average increase across that period in all four groups. In contrast, on the HbTBI foot, the placebo group’s average TBI increased from baseline to Visit 3, but the other groups’ average TBIs decreased. Across both feet, the placebo group stands out with slightly smaller minimum, and noticeably greater maximum, change values. The standard deviations were similar on both feet across all of the groups.

\(^{10}\) ANCOVA is regarded as the optimal method for analysing pre- and post-intervention data in RCTs, and, by implication, experiments, when covariates are employed; in most situations it is demonstrably superior to other methods such as ANOVA based on difference or percentage change scores, which should be used with caution (Twisk & Proper, 2004; Van Breukelen, 2006; Vickers & Altman, 2001; Zhang et al., 2014).
Table 5.34

Difference Scores for Both Feet from Baseline to Visit 3 (N = 100)

<table>
<thead>
<tr>
<th>Foot/Group</th>
<th>Number</th>
<th>Means Baseline</th>
<th>Means Visit 3</th>
<th>Differences (Baseline–Visit 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>LbTBI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low GTN 33</td>
<td>.466</td>
<td>.529</td>
<td>.063</td>
<td>.187</td>
</tr>
<tr>
<td>High GTN 24</td>
<td>.518</td>
<td>.621</td>
<td>.104</td>
<td>.181</td>
</tr>
<tr>
<td>Placebo 22</td>
<td>.466</td>
<td>.582</td>
<td>.116</td>
<td>.173</td>
</tr>
<tr>
<td>Control 21</td>
<td>.487</td>
<td>.543</td>
<td>.057</td>
<td>.134</td>
</tr>
<tr>
<td>HbTBI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low GTN 33</td>
<td>.571</td>
<td>.564</td>
<td>-.007</td>
<td>.198</td>
</tr>
<tr>
<td>High GTN 24</td>
<td>.617</td>
<td>.600</td>
<td>-.017</td>
<td>.162</td>
</tr>
<tr>
<td>Placebo 22</td>
<td>.571</td>
<td>.620</td>
<td>.049</td>
<td>.167</td>
</tr>
<tr>
<td>Control 21</td>
<td>.607</td>
<td>.591</td>
<td>-.017</td>
<td>.166</td>
</tr>
</tbody>
</table>

The above differences were the basis for the ANCOVAs, with adjustments made as a result of covariate contributions. The TBIs on the LbTBI foot at baseline were used as one covariate.\(^{11}\) The TBIs of the HbTBI foot at Visit 3 were also chosen as a covariate on the assumption that there would be a GTN effect on the treated side only (refer to Section 5.3.7);\(^{12}\) furthermore, it was assumed that both feet would be equally influenced by other variables affecting TBIs such as seasonal temperature, ambient temperature, recent physical activity, recent caffeine and food intake, smoking, pain or discomfort, emotional state, and central blood pressure, so using the TBIs of the HbTBI foot at Visit 3 as a covariate would permit those influences to be captured effectively.

Additional covariates were not chosen because, apart from TBIs, all other plausible variables in the data set were either categorical (many were dichotomous) or had failed to demonstrate associations with LbTbIs at baseline in this sample.

\(^{11}\) Using baseline values on the dependent variable as a covariate in ANCOVA is regarded as standard practice and as likely to produce the most efficient outcomes (see Van Breukelen, 2006; Vickers, 2001).

\(^{12}\) One determinant of the validity of ANCOVA is that covariates should be independent of the treatment effect (Portney & Watkins, 2009).
Both of the covariates satisfied the two main criteria for covariates associated with correlations. First, as can be seen in Table 5.35, both the LbTBIs at baseline and HbTBIs at Visit 3 had satisfactorily high correlations with the dependent variable (LbTBI at Visit 3), $r_s = .55$ and $r_s = .74$ respectively.\textsuperscript{13} Second, both variables had a sufficiently low correlation with each other, $r_s = .53$ (see Table 5.35).\textsuperscript{14}

Table 5.35

\textit{Correlations Between LbTBI and HbTBI TBIs at Baseline and Visit 3}\textsuperscript{a}

<table>
<thead>
<tr>
<th>TBI</th>
<th>HbTBI, baseline</th>
<th>LbTBI, Visit 3</th>
<th>HbTBI, Visit 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>LbTBI, baseline</td>
<td>.71</td>
<td>.55</td>
<td>.53</td>
</tr>
<tr>
<td>HbTBI, baseline</td>
<td>.47</td>
<td>.51</td>
<td></td>
</tr>
<tr>
<td>LbTBI, Visit 3</td>
<td></td>
<td>.74</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} Correlations are Spearman’s rank order coefficients. $N = 100$ for all entries, and all are significant at the $<.001$ level. Entries associated with covariates are in larger font size and bolded.

Prior to conducting the ANCOVAs, several required assumptions were known to have been satisfied. These were that the dependent variable and covariates could be regarded as equal interval measurements, that there were no outliers in the data, that the covariates were not related to the independent variable (as indicated above, GTN appeared not to affect the HbTBI foot), and that each observation was independent. Preparatory analyses using scatterplots and correlation coefficients indicated that the relationships between the covariates and dependent variable were linear. Homogeneity of regression (equality of slopes) and homogeneity of variance across groups were assessed in the process of conducting the ANCOVAs.

\textsuperscript{13} According to Plichta and Kelvin (2013), correlations between the dependent variable and covariates greater than .30 are acceptable, and Portney and Watkins (2009) state that ANCOVA is most effective when correlations between the dependent variable and covariates are greater than .60.

\textsuperscript{14} Tabachnick and Fidell (2012) and Stevens (2009) recommend that the correlation(s) between covariates should be “moderate”, and Bryman and Cramer (2011) state that correlations greater than .80 are unacceptably high.
5.3.8.1.1 ANCOVAs excluding the placebo group

The requirement that the dependent variable be approximately normally distributed within each category of the independent variable may have been unmet for the placebo group. That group had a platykurtic distribution on the LbTBI foot at Visit 3 (kurtosis = –1.2), as indicated by the histogram in Figure 5.8. The extent of platykurtosis might be exaggerated in that figure, however, because of the way in which SPSS generates histograms, and that possibility is highlighted by the Kolmogorov-Smirnov and Shapiro-Wilk tests of normality both being insignificant, $p = .200$ and $p = .224$ respectively. Nevertheless, because of the conflicting evidence concerning the normality of its distribution on the dependent variable, the placebo group was initially excluded from the baseline–Visit 3 ANCOVAs as a conservative measure to ensure their fidelity.

The first ANCOVA was therefore conducted using data from only the remaining three groups ($n = 78$). This analysis suggested that, although homogeneity of regression slopes and homogeneity of variance across groups were satisfactory, there were no significant differences between the four groups, $F(2, 73) = 1.60, p = .209$. Consequent to this lack of statistical significance, no pairwise post hoc comparisons were conducted despite a priori hypotheses having been proposed.

However, because of concerns about the quality of data from the TBI-inconsistent participants (see Section 5.3.4.1), a subsequent ANCOVA was conducted, again excluding the placebo group members, but also excluding any of the 14 participants...
from the other three groups whose TBIs had earlier been identified as problematically unstable. All assumptions for ANCOVA were still met.

In this analysis, the omnibus $F$ value indicated that there were significant differences in TBIs among at least some of the three groups, $F(2, 62) = 3.45, p = .038$. The associated partial eta squared ($\eta_p^2$) of .100 revealed that 10% of the variance in TBI scores at Visit 3 was accounted for by the intervention. This might be regarded as a small to medium effect according to Bakeman (2005).

Post hoc pairwise comparisons using Bonferroni corrections with bootstrapping\(^{15}\) indicated that TBI values at Visit 3 had increased to a greater extent in the high GTN group relative to both the low GTN group, $p = .024$, and the control group, $p = .012$. The low GTN group was not significantly different from the control group, $p = .543$.

Details about the two post hoc comparisons that attained statistical significance, with relevant differences between the means as well as significance levels, effect sizes,\(^{16}\) and confidence intervals, are provided in Table 5.36. The effect sizes of .623 and .772 correspond to Cohen’s $d$ (Cohen, 1988) in this context and therefore can both be regarded as moderate.

Table 5.36  

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Difference between means</th>
<th>Sig. (2-tailed)</th>
<th>Effect size(^b)</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>High GTN</td>
<td>Low GTN</td>
<td>.071</td>
<td>.024</td>
<td>.623</td>
<td>.007</td>
<td>.130</td>
</tr>
<tr>
<td>High GTN</td>
<td>Control</td>
<td>.088</td>
<td>.012</td>
<td>.772</td>
<td>.016</td>
<td>.156</td>
</tr>
</tbody>
</table>

\(^a\) These entries are based on data from only the TBI-consistent participants.  
\(^b\) Effect sizes are larger than indicated by the $\eta_p^2$ of .100 in the full ANCOVA because only significant pairwise comparisons are involved in these comparisons, whereas not all of the comparisons in the full ANCOVA were significant.

\(^{15}\) All post hoc pairwise comparisons in the ANCOVAs were conducted with bootstrapping, set at 500 samples, to compensate for some of the stringency associated with avoidance of Type I errors in Bonferroni corrections.  
\(^{16}\) Effect sizes for post hoc pairwise comparisons were calculated by dividing the absolute difference between the two relevant adjusted means by the square root of the mean square error.
The original and adjusted means for the three groups are shown in Table 5.37.

Table 5.37

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>Original</th>
<th>Adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low GTN</td>
<td>.475</td>
<td>.510</td>
<td>.537</td>
</tr>
<tr>
<td>High GTN</td>
<td>.528</td>
<td>.636</td>
<td>.608</td>
</tr>
<tr>
<td>Control</td>
<td>.498</td>
<td>.530</td>
<td>.520</td>
</tr>
</tbody>
</table>

These entries are based on data from only the TBI-consistent participants.

5.3.8.1.2 ANCOVAs including the placebo group

Two additional ANCOVAs were conducted in which the placebo group was included in case the outcomes proved to be both valid and even more informative. The same two covariates were selected, and they continued to meet all necessary criteria. Furthermore, all assumptions for conducting ANCOVA were also met—assuming that the placebo group’s distribution on the LbTBI was sufficiently normal.\(^\text{17}\)

In the first of these ANCOVAs, all participants from the four groups were included. This analysis suggested that, although homogeneity of regression slopes and homogeneity of variance across groups were satisfactory, there were no significant differences between the four groups, \(F(3, 94) = 1.14, p = .338\).

Again, however, because of concerns about the quality of data from the TBI-inconsistent participants a subsequent ANCOVA was conducted excluding the 14 participants whose TBIs had earlier been identified as unstable. All assumptions for ANCOVA were still met, both prior to, and in the course of, conducting the analysis.

\(^\text{17}\) Olejnik & Algina (1983) demonstrated that parametric ANCOVA is robust to violations of either normality or homoscedasticity, but not to concurrent violations of both. Therefore, inclusion of the placebo group with a non-normal distribution would have been problematic only in the absence of homogeneity of variance—and homogeneity of variance was later confirmed in the Levene tests associated with the ANCOVAs.
This analysis yielded a result that approached statistical significance, \( F(3, 80) = 2.52, p = .064 \). The associated \( \eta_p^2 \) of .086 indicates that 8.6% of the variance in TBI scores at Visit 3 was accounted for by the intervention. Given the earlier finding of significant differences between some groups, and despite the insignificant omnibus F value, pairwise comparisons were conducted in the knowledge that appropriate protection could be applied against Type I errors.\(^{18}\)

Therefore, the post hoc pairwise tests were again conducted with Bonferroni corrections, and these were again applied in conjunction with bootstrapping. They indicated, as before, that TBI values on the LbTBI foot had increased significantly from baseline to Visit 3 in the high GTN group relative to both the low GTN group, \( p = .046 \), and the control group, \( p = .020 \). The placebo group’s TBIs were not significantly different from those of the low GTN group, \( p = .601 \), the high GTN group, \( p = .144 \), or the control group, \( p = .331 \).

Details about the two post hoc comparisons that attained statistical significance, with relevant differences between the means as well as significance levels, effect sizes, and confidence intervals, are provided in Table 5.38. The effect sizes of .676 and .822, which correspond to Cohen’s \( d \) (Cohen, 1988), can be regarded as moderate and large respectively.

The original and adjusted means for the four groups are provided in Table 5.39 and are charted in Figure 5.9. There it is evident that, although there were no significant differences between them, the placebo group’s TBIs increased slightly more than did the TBIs of both the low and high GTN groups (with increases of 0.095, 0.060, and 0.081 respectively).

\(^{18}\) Taylor (2011) has pointed out that “many of the well-known post hoc procedures (the Tukey, Scheffé and the Bonferroni procedures, for example) control Type I error at the designated level in the absence of a significant overall result [i.e., significant omnibus F value in the ANCOVA]. Further, by using them only if the overall test is significant, the researcher is being conservative, and unduly reducing the power of the methods”.
Table 5.38

**Significant Pairwise Comparisons for Four-Group ANCOVA of Baseline vs Visit3 TBIs**

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Difference</th>
<th>Sig. (2-tailed)</th>
<th>Effect size</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>High GTN</td>
<td>Low GTN</td>
<td>.073</td>
<td>.046</td>
<td>.676</td>
<td>.006 - .141</td>
</tr>
<tr>
<td>High GTN</td>
<td>Control</td>
<td>.090</td>
<td>.020</td>
<td>.822</td>
<td>.017 - .158</td>
</tr>
</tbody>
</table>

*These entries are based on data from the 86 TBI-consistent participants.*

*Effect sizes are larger than indicated by the \( \eta^2 \) of .086 in the full ANCOVA because only significant pairwise comparisons are involved in these comparisons, whereas some comparisons across all groups in the full ANCOVA were not significant.*

Table 5.39

**Original TBI Means on the LbTBI Foot at Baseline, and Original and Adjusted TBI Means on the LbTBI Foot at Visit 3 in Four-Group ANCOVA**

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>Original</th>
<th>Adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low GTN</td>
<td>.475</td>
<td>.510</td>
<td>.535</td>
</tr>
<tr>
<td>High GTN</td>
<td>.528</td>
<td>.636</td>
<td>.609</td>
</tr>
<tr>
<td>Placebo</td>
<td>.458</td>
<td>.551</td>
<td>.553</td>
</tr>
<tr>
<td>Control</td>
<td>.498</td>
<td>.530</td>
<td>.519</td>
</tr>
</tbody>
</table>

*These entries are based on data from the 86 TBI-consistent participants.*
Figure 5.9. Charts showing means at baseline and adjusted means on the LBTBI foot at Visit 3 for the four groups. These charts are based on data from the 86 TBI-consistent participants only.

5.3.8.2 Effect of GTN from baseline to Visit 7

Table 5.40 contains the descriptive statistics relevant to the differences in TBIs from baseline to Visit 7. Again, these are based on entries in Table 5.30 in Section 5.3.4.3. On the LbTBI foot there were increases across that period in all groups except for the control group, which fell slightly. The placebo group had the greatest mean increase (0.110)—noticeably greater than was the increase of either intervention group—and a much higher maximum score (0.65). On the HbTBI foot, although the low GTN and placebo groups’ TBIs increased slightly from baseline to Visit 7, the high GTN and control groups’ average TBIs decreased slightly. The standard deviations were slightly larger on both feet for the placebo group. Again, these differences were the basis for the ANCOVAs, with adjustments made because of covariate contributions.
The dependent variable, LbTBI TBIs at Visit 7, was normally distributed within each of the four groups, so all groups were included in these analyses. The covariates chosen paralleled those chosen for the baseline–Visit 3 ANCOVAs. In this case they were the LbTBI at baseline (again) and the HbTBI at Visit 7. Correlations between the covariates and the dependent variable are provided in Table 5.41. They indicate that the covariates were, as in the earlier analyses, appropriately correlated with the dependent variable and with each other.

Table 5.41

Correlations Between LbTBI and HbTBI TBIs at Baseline and Visit 7

<table>
<thead>
<tr>
<th>TBI</th>
<th>HbTBI, baseline</th>
<th>LbTBI, Visit 7</th>
<th>HbTBI, Visit 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>LbTBI, baseline</td>
<td>.71</td>
<td>.52</td>
<td>.44</td>
</tr>
<tr>
<td>HbTBI, baseline</td>
<td>.44</td>
<td>.47</td>
<td>.69</td>
</tr>
</tbody>
</table>

a Correlations are Spearman’s rank order coefficients. N = 100 for all entries, and all are significant at the < .001 level. Entries associated with covariates are in larger font size and bolded.
Prior knowledge about the data (including them being at the equal interval level and containing no outliers) and preparatory analyses (comprising checks on homogeneity of regression slopes and homogeneity of variance across groups) ensured that all other assumptions that had been met for the baseline–Visit 3 ANCOVAs were again satisfied.

An initial ANCOVA using data from the total sample (100 participants) comparing the four groups’ scores at baseline and Visit 7 approached statistical significance, $F(3, 94) = 2.39, p = .074$, suggesting that there might be some significant differences between the groups. This was not pursued in post hoc comparisons because data from the TBI-inconsistent participants were included and thus more definitive, and accurate, outcomes might have been obscured.

Instead, a subsequent ANCOVA excluding the 14 participants whose TBIs had been identified as unstable was conducted. This analysis also yielded an $F$ value that marginally failed to reach statistical significance, $F(3, 80) = 2.56, p = .061$. The associated $\eta^2_p$ of .088 from this analysis nevertheless indicated that 8.8% of the variance in TBI scores at Visit 7 could be accounted for by the intervention.

In order to avoid committing Type II errors, particularly keeping in mind the small sample sizes, the earlier reluctance to alter any of the data in a way that would favour the research hypothesis (even if doing so might have been partly defensible), and the presence of a priori hypotheses, these results were regarded as indicating that significant differences in TBIs might be present between at least some of the groups. Furthermore, given that an insignificant omnibus $F$ value should not preclude conducting pairwise comparisons in the presence of appropriate protection against Type I errors, those comparisons were again produced using Bonferroni corrections with bootstrapping. They indicated that TBI values had increased significantly from baseline to Visit 7 in the low GTN group relative to the control group, $p = .048$, in the high GTN group relative to the control group, $p = .044$, and in the placebo group relative to the control group, $p = .006$. There was no difference between the low and high GTN groups, $p = .792$, and the placebo group was not significantly different from either the low GTN group, $p = .186$, or the high GTN group, $p = .347$. 
Details about the three post hoc comparisons that attained statistical significance, with relevant differences between the means as well as significance levels, effect sizes, and confidence intervals are provided in Table 5.42. The effect sizes, ranging from .531 to .932, may be regarded as moderate for the two GTN groups relative to the control group, and large for the placebo group relative to the control group.

Table 5.42

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Difference between means</th>
<th>Sig. (2-tailed)</th>
<th>Effect size&lt;sup&gt;b&lt;/sup&gt;</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low GTN</td>
<td>Control</td>
<td>.065</td>
<td>.048</td>
<td>.531</td>
<td>.006 - .131</td>
</tr>
<tr>
<td>High GTN</td>
<td>Control</td>
<td>.075</td>
<td>.044</td>
<td>.662</td>
<td>.005 - .149</td>
</tr>
<tr>
<td>Placebo</td>
<td>Control</td>
<td>.114</td>
<td>.006</td>
<td>.932</td>
<td>.045 - .188</td>
</tr>
</tbody>
</table>

<sup>a</sup> These entries are based on data from the 86 TBI-consistent participants.

<sup>b</sup> Effect sizes are larger than indicated by the $\eta^2_p$ of .088 in the full ANCOVA because only significant pairwise comparisons are involved in these comparisons, whereas some comparisons across all groups in the full ANCOVA were not significant.

The original and adjusted means for the four groups are provided in Table 5.43 and charted in Figure 5.10. According to the baseline and adjusted means there, although the TBIs of the LbTBI foot decreased over the six-month timespan for participants in the control group, and the TBIs of both intervention groups increased, the TBIs increased even more, if not significantly so in the ANCOVA, for the placebo group than they did for either of the intervention groups.

Table 5.43

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>Original</th>
<th>Adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low GTN</td>
<td>.475</td>
<td>.520</td>
<td>.530</td>
</tr>
<tr>
<td>High GTN</td>
<td>.528</td>
<td>.560</td>
<td>.540</td>
</tr>
<tr>
<td>Placebo</td>
<td>.458</td>
<td>.574</td>
<td>.579</td>
</tr>
<tr>
<td>Control</td>
<td>.498</td>
<td>.463</td>
<td>.465</td>
</tr>
</tbody>
</table>

<sup>a</sup> These entries are based on data from the 86 TBI-consistent participants.
For the baseline to Visit 3 timespan, in the initial analyses when the placebo group was removed because its distribution’s departure from normality on the dependent variable might have been too great, the first ANCOVA disconfirmed the expectation that the GTN patches would produce an increase in TBIs in the low TBI pressure foot of the two intervention groups. However, a different picture emerged when the TBI-inconsistent people were also removed from the analysis. It then became apparent that there was a significant increase in TBIs of the LbTBI foot for participants in the high GTN group relative to the TBIs of participants in both the low GTN and control groups. When the placebo group was included in the analyses, similar results were obtained: The high GTN participants had greater TBI increases than did participants in both the low GTN and control groups. An additional outcome emerged when the TBI-inconsistent participants were excluded, namely that the placebo group did not differ significantly from any of the other three groups.
The baseline to Visit 7 ANCOVAs marginally failed to attain statistical significance on the omnibus $F$ values. This occurred both when the full complement of participants, and when only the 86 TBI-consistent participants, were included. However, given the proximity to statistical significance attained in both ANCOVAs and the possibility of committing Type II errors in the presence of small samples and a priori hypotheses, post hoc comparisons were conducted with appropriate protection against Type I errors. Those comparisons revealed that the low GTN, high GTN, and placebo groups did not differ significantly from each other, but all had significantly higher TBIs on their LbTBI feet compared with the control group.

5.3.8 Responders and non-responders to GTN

Looking for patterns in demographics and health status that might predict a response to GTN, it was decided to conduct the following additional analyses. Both low and high GTN groups were combined based on their data at Visit 7. They were then divided into responders and non-responders. Responders were defined as people whose TBIs on the LTBI foot had increased by more than 0.100 from baseline to Visit 7. This group comprised 23 participants (14 from the low GTN group and nine from the high GTN group). The non-responders were defined as those whose TBIs on the LbTBI foot had decreased at Visit 7 relative to baseline. This group also comprised 23 participants (13 from the low GTN group and 10 from the high GTN group). The remaining 11 treatment group participants had identical TBIs on their LbTBI foot at baseline and Visit 7 or had increased by no more than 0.100 across that time period. These 11 stable participants are omitted from the analyses below.

A small number of differences between the two groups emerged. People who had diabetes were significantly less likely to respond well to the GTN treatment $\chi^2(1) = 4.39, p = .036$. Of the 27 people who had diabetes, 17 did not respond well to GTN whereas 10 did. Conversely, of the 19 people who did not have diabetes, 13 responded well to GTN whereas six did not.

People who had smoked for more than 10 years were also less likely to respond well to GTN treatment, $\chi^2(1) = 4.39, p = .036$. Of the 19 people who had smoked for more than 10 years, six responded well to GTN, whereas 13 did not. Conversely, of the 27
people who had smoked for 10 years or less, or not at all, 17 responded well to GTN whereas 10 did not. A chi-square analysis comparing those who had never smoked with those who had smoked at any time in their lives favoured those who had never smoked in terms of GTN response, but failed to reach significance, $p = .109$.

Finally, people with lower levels of mobility were less likely to respond well to GTN, $\chi^2(1) = 6.77$, $p = .009$. Of the nine people with a medium level of mobility, eight did not respond well to GTN whereas one did. Conversely, of the 37 people who had high/normal mobility, 22 responded well to GTN whereas 15 did not.

Comparisons between participants below and above the recommended levels of alcohol intake indicated no significant differences. Those on antihypertensive medication tended to respond to GTN, but not significantly more so, $p < .075$. People in the low (nonresponder) group had higher HbA1c scores (4.73 vs 2.76), but this failed to reach statistical significance, $t(45) = 1.84$, $p = .073$. A summary of the health and demographic characteristics that had significant relationships with GTN responders and nonresponders is presented in Table 5.44.

Table 5.44  
*Health Status Conditions Distinguishing GTN Responders and Nonresponders*

<table>
<thead>
<tr>
<th>Health status</th>
<th>Nonresponders</th>
<th>Responders</th>
<th>$\chi^2$</th>
<th>df</th>
<th>Sig.$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes</td>
<td>17</td>
<td>10</td>
<td>4.39</td>
<td>1</td>
<td>.036</td>
</tr>
<tr>
<td>No diabetes</td>
<td>6</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smokers&gt;10 years</td>
<td>13</td>
<td>6</td>
<td>4.39</td>
<td>1</td>
<td>.036</td>
</tr>
<tr>
<td>Nonsmokers or &lt;10 years smoking</td>
<td>10</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mobility—Medium: ADLs only</td>
<td>8</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mobility—High/normal</td>
<td>15</td>
<td>22</td>
<td>6.77</td>
<td>1</td>
<td>.009</td>
</tr>
</tbody>
</table>

$^a$ 2-tailed test.
5.4 Discussion

This section contains a discussion of the issues that relate directly to the experiment. First, the methods used are considered, with discussions about independence versus non-independence of analyses, intention to treat, and the removal of the TBI-inconsistent people from the main analyses. Group similarities and differences are discussed, then the variability of TBIs is addressed, including discussion of some issues that affect TBI measurement accuracy. Significant relationships of TBIs with demographic and health variables are then brought forward for consideration.

Issues concerning the results of the GTN intervention are then discussed. These include the associations of GTN with TBIs based on the ANCOVAs. Side effects of GTN are also discussed, providing a window onto the topic of local versus systemic effects. Unexpected placebo outcomes are addressed here. This section concludes with an acknowledgement of the limitations of the study and a summary of the key points.

A more general discussion, including implications for enhancing clinical practice with this knowledge, and further directions and recommendations for future research not directly specific to this experiment, occur in the next and final chapter, Chapter 6.

5.4.1 Methodological considerations

This research encountered some issues in obtaining the most accurate and valid measures. These are addressed in this section, with justification for the solutions used in this enquiry, often accompanied by recommendations for future research.

The primary outcome variable for this trial was the toe brachial index (TBI). Although this measure is recommended in international guidelines (see Appendix B), there is little procedural consensus in the literature for measuring TBIs. Furthermore, little information is available about their characteristics, and normal and normative values are still being established. Therefore it was necessary to explore the nature of TBIs in some depth to determine the most valid approach to analysis. In doing so, a number of characteristics concerning these were revealed. Investigation of these characteristics proved to be essential for the validity of the analyses and interpretations reported in this chapter.
5.4.1.1 Measurement issues

5.4.1.1.1 Independence versus nonindependence of measures

The data were analysed with sensitivity to the issues of independence versus nonindependence of measures. As Menz (2004) has pointed out, the data from two separate feet cannot usually be doubled and used as two separate data points without the risk of spurious conclusions. In this study, the problem could have been compounded by regarding the data taken on a number of different occasions as having come from different people rather than being data taken successively from the same group of people. Therefore, except when it was conceptually sound to base analyses on data obtained from both feet, or from more than one occasion, all measurements refer to single independent measures in which data from a single foot on a single occasion were analysed from each participant.

5.4.1.1.2 Intention to treat versus per protocol

The distinction of intention to treat versus per protocol is acknowledged here with awareness that the data were analysed on a per protocol basis, reflecting the actual interventions performed in the subjects that were retained in the trial. This approach was deemed superior to analyses based on intention to treat because of pragmatic considerations related to the aim and methodology of this study. The 22 participants who failed to complete the study usually discontinued very early in the process. Following the intention to treat method of analysis would have resulted in those clients’ data being predominantly their baseline readings which would have had to be extrapolated forward through to the later measurement points of the study without modification. This would have had a dampening effect on any positive results from the people who were actually exposed to the intervention and may have led to Type II errors.

The intention to treat protocol measures compliance with the requirements of a study. In this study, none of the people who discontinued cited problems with the intervention as the reason for withdrawal. The most common reasons for withdrawal were associated with ill health not related to the patch intervention. In fact, compliance with the routines of the study was extremely high, with very little deviation from 100% adherence to patch application and attendance at appointments for nearly all of the participants.
Information about the participants lost to follow-up was presented in Section 5.3.1.2 and Appendix K. The percentages lost from each group are roughly equal, with 20%, 22%, 12%, and 18% respectively from the groups’ original numbers. Dettori (2011) has indicated that any loss to follow-up greater than 20% could be problematic for the validity of results from a study. It is important, therefore, to inspect the reasons for the discontinuation, particularly checking whether adverse effects of the intervention might have been responsible. It can be seen from the details provided in Appendix K that the reasons for discontinuing were not related to adverse effects from GTN. Therefore, although the dropout rates were equal to or greater than 20% for the two intervention groups, in both cases the attrition was primarily associated with the greater levels of preexisting underlying pathology.

5.4.1.1.3 Data adjustments

It was important for the validity of the study to make each of the changes that were made to the data that are described in Sections 5.3.2, 5.3.3, and 5.3.4 of this chapter. These included the initial data cleaning with management of extreme outliers as detailed in Table 5.12. Other tables, from Table 5.23 to Table 5.28 cover the data management issues, ending with evaluation of the differences in TBI according to cuff size.

The changes in the data management process led to noticeable alterations in both the order and magnitude of the baseline data across the four groups, revealing that without these adjustments the results could have been affected in ways that led to erroneous conclusions.

5.4.1.1.4 Removal of TBI-inconsistent participants

It was not only justifiable, but apparently necessary, to remove the TBI data of the 14 most TBI-variable participants from some of the analyses. The table showing details of the medical history of each of these excluded people (Table 5.26) demonstrated primary or secondary physiological reasons for fluctuations of BPs and therefore TBIs. These fluctuations, concomitant with particular pathologies, do need to be expected and
acknowledged in practice and research on these populations. However, in order to answer the research questions, these adjustments were valid.

### 5.4.1.2 Group similarities and differences

For valid comparison between the groups, it was important that the groups were well matched for their cardiovascular (CV) conditions of the participants and their CV medications. Significant differences between the four groups were less important to the overall study foundations than between the intervention and control categories. Only one significant difference was found between these two categories, namely that “other” medical conditions (non-CV) were more prevalent in the intervention groups. It was concluded, therefore, that the group comparisons were indeed valid in the context of this project.

However, when the differences between the four individual groups were analysed, several significant differences emerged, as seen in Table 5.10. These differences between groups invariably favoured the nonintervention groups because they indicated greater pathology in the intervention groups compared with the nonintervention groups.

These differences were in regard to factors that are relevant to the study outcomes, namely smoking history, history of cardiovascular surgery, and antihypertensive use. The differences that approached significance are listed in Table 5.11 and show the same strong pattern of pathology in one or other of intervention groups compared with one or other of the control groups.

Despite this pervasive bias for pathology at greater levels in the intervention groups, the baseline TBIs were evenly distributed across each group as shown in Table 5.29, Section 5.3.4.3 after the final adjustments for aberrant data and cuff size.

### 5.4.1.3 Obtaining accurate TBIs

The accuracy of TBIs is affected by several conditions, and this difficulty is acknowledged in this study. The protocol in Appendix C lists the conditions that affect TBIs, and a number of considerations are offered in regard to enhancement of accuracy. The TBI is dependent not only on the toe pressure but also on the denominator of the index, the brachial blood pressure, which undergoes known variations associated with several intrinsic
and extrinsic factors. The recommendation of Perez-Martin et al. (2012) to take three measures on each monitoring opportunity was assumed as useful practice due to the variability that existed at times between the measures. Several authors recommend that toe pressure measurements be repeated on serial occasions to build the most accurate representation of pedal arterial supply (Høyer, Sanderman, & Petersen, 2013b; Sadler, Chuter, & Hawke, 2013).

Several issues, particularly fluctuations in brachial blood pressure (BP), are fundamentally implicated in TBI variability and are influenced by the factors listed in Table 4.2 in Section 3.4.2.3. Among the conditions noted to increase BP are laboured breathing, with values of 5–8 mm Hg increases documented, and pain, causing 10–30 mm Hg increases. Even the level of discomfort associated with a full bladder is known to increase brachial BP by 10–15 mm Hg. These fluctuations of TBI measurements need to be considered when diagnostic and research conclusions are sought.

5.4.1.3.1 Warming

Issues of warming, best summarised by Høyer et al. (2013b), were not explored in this study, although recommendations were followed to use a commercially available foot warmer when pressures were unprocurable (Cloete, 2009). TBIs are higher when feet are warmed. Procedures of warming require standardisation with further research. In the review by Høyer et al. (2013b), studies were classified by whether or not warming was used. A less variable and higher average for normal TBIs was estimated from the averaged values of five studies to be 0.90 with warming. Without warming, an average of 0.77 from three studies was obtained. Warming is recommended to give the most accurate results. Among the TBI systems available internationally, one, the Swedish Peri 5000, addresses this with inbuilt standardised warming around the location of the sensor contact.

A decision was made for this study not to use pre-test warming except when TBIs were unprocurable and skin temperatures were below 20 °C to most closely duplicate the likely clinical situations in which TBIs would be taken in practice. Warming for all participants would have given higher, less variable, and more accurate results according to the comparisons of studies by Høyer et al. (2013b).
5.4.1.3.2 Positioning

Unless the test subject can be positioned lying supine, with the feet at the same level as the heart, the results for toe pressure will be significantly inflated. Unfortunately, lying flat is difficult for some people due to associated medical conditions in particular respiratory and musculoskeletal problems. It was noted early in the study that although the act of reclining is resisted by many people, instead of attempting to recline them in a hydraulic chair from a seated position, offering two pillows on a flat surface enabled most people to lie flat, even those who could not initially be reclined to flat from a seated position.

5.4.1.3.3 Cuff size issues

Cuff size has significant implications for measurement results, and this has been underappreciated in the literature to date. Issues with choosing the correct cuff size have come to light recently since innovations have introduced automated inflation with twin occlusion and sensor cuffs. The large, 2.5 cm cuff, which is the most accurate and the default measure, fits only 54% of the population (based on the findings of this research) when a twin cuff system is used. In light of the best evidence available at the time of planning this study (Pålhlsson et al., 2007), an estimation of a 0.15 greater small cuff threshold was made for a comparable indication of a cut-off for pathology. Therefore, for inclusion in the study using a small cuff, a threshold TBI value of 0.80 (0.65 +0.15) was chosen. In light of the results of the data collected for this experiment, where 0.046 on the LbTBI was found to be the difference between measurements made using the large and small cuffs, and 0.083 on the HbTBI feet, 0.060 was subtracted from all small cuff measurements to give them a baseline for comparison with measurements taken with a large cuff.

The validity of the choice of the small cuff cut-off value of .80 in the inclusion criteria was cast into doubt in retrospect by the result of an average difference in TBIs measured with the large and small cuffs of only .06 determined from this study. In hindsight, the larger difference reported in the literature (Pålhlsson et al., 2007) from a very small sample (n=20) may result from the use of small cuffs on a large toe, as the same toes were tested.
with different sized cuffs. In the present study, by contrast, the cuffs were always chosen to fit the toe accurately.

The large cuff TBI value in normal populations without warming is estimated at 0.77 as cited in the review article on this topic (Høyer et al., 2013b). Taking into consideration that small cuffs were associated with higher measures in the present study by an average of 0.06, with a small cuff, the threshold for normal is calculated by adding these two values to equal .83, so in the protocol in this study, test subjects qualifying for inclusion with a small cuff TBI of $\leq .80$ would still have fallen under the threshold of subnormal toe pressures.

The variability using the small cuff on small sized toes in this study was not significantly greater than the variability of the large cuff readings. A preliminary investigation in the literature suggests that smaller cuffs may exhibit higher variability (Påhlsson et al., 2007). This may mean that when a small cuff is used on a large toe, (as in the Påhlsson et al. study specifically testing various cuff sizes on the toes) variability increases from the increased cutting in and the resultant inappropriate vessel compression. The small cuffs used in the present study were used only when the default large size would not fit on the toe.

5.4.2 Results concerning TBIs

In this section there are six subsections dealing with the details of results with TBIs. Issues that pertain to variability, validity of measures, and the associations between health and demographic variables are included here.

5.4.2.1 TBI variability/inconsistency

Three TBI readings that were taken on each foot at each measurement episode varied noticeably in 29% of people (see Tables 5.26 and 5.27). In the experiment reported in this chapter, the lowest reading of three was chosen as the least likely to be subject to artefactual influences and was the therefore the only one used in the analyses from Section 5.3.3 onwards. This strategy runs counter to the protocol of Perez-Martin et al. (2010), who recommended ignoring the first reading and averaging the second two. Bonham (2010) used and recommended 2 readings. Where duplicate measures are not mentioned in other
literature, it is assumed to be based on single TBI readings, and results may therefore be less valid and reliable.

As outlined in Section 5.3.4.1 and detailed in Table 5.27 with information about 15 participants who were retained in the analysis but who had high variability in their TBIs, it is clear that people with PAD are likely to have significant co-morbidities that are related to fluctuations in TBIs. In this subgroup of 15, 8 had a history of cardiac disease, 7 had respiratory diseases, 6 had diabetes, and 3 had severe chronic pain which may each be associated with variability in pedal vascular supply.

5.4.2.2 Number and order of TBI readings

The order of the readings did not make a significant difference to the TBIs. However, the second and third readings were slightly closer to each other than were any other pair of readings, with means of the difference ranging between .054-.069. The first was slightly more discrepant than the others with its maximal differences being from the third, with mean differences readings ranging between .073-.100. This gives rise to the recommendation that two readings would suffice if they are similar to each other, but if different, then a third reading should be taken.

Although the average discrepancies between the three readings were not very different from each other in this study, the extent of some discrepancies indicates that outlier management, and perhaps other forms of data cleaning were justified, certainly for the data set of this study, but also for other data sets used in TBI research. It also flags a warning for practitioners not to rely on a single TBI reading, and as a pointer for the need for data management in future research.

5.4.2.3 Differences between feet

In addressing the question of how different the measures are for each foot of the same person, the results demonstrate the apparent paradox that although the two feet are significantly correlated, they can also be significantly different from each other. Furthermore, the lack of high correlations suggests that the TBIs of individuals may vary significantly at certain times (see the next section).
5.4.2.4 Temporal stability of TBIs

The control group was the only group in which assessment of temporal stability could be validly conducted, and both feet changed to the same degree. TBIs moved by at least 0.10 for 50% of control group participants from baseline over both of the time periods studied (2 months and 6 months). TBIs changed by 0.20 or more in 25% of those participants. However, these changes were analysed as not significant according to several tests of correlation and differences. For 43%, the LbTBI and the HbTBI reversed their places. The fluctuation in the TBIs of the control group provides an indication of the degree of inherent instability in toe pressures. This point is followed up in the general discussion with further links to the possible pathology indicated by interfoot differences.

5.4.2.5 Associations of health and demographic variables with TBIs

5.4.2.5.1 Age and TBIs

Contrary to expectations that there would be an inverse relationship between age and TBIs, age was not related to TBIs in this sample. The participants had a mean age of 69.8 years and standard deviation of 9.5. Therefore there was not a spread of ages wide enough to make a valid conclusion regarding relationships with age in this study population.

5.4.2.5.2 Smoking and TBIs

There was a strong association with current smokers and low TBIs, in contrast to that of all exsmokers, including those with a long-term smoking history), but no significant relationship was found, surprisingly, between TBIs and years smoked. This could be linked with the high proportion of people who had smoked at some time, which was 44% of the sample.

This might mean that smoking cessation at any time is of value to boost toe pressures. This could be used to support health behaviour promotion by providing evidence of the immediate benefits of smoking cessation to the peripheral circulation.

There are difficulties in measuring the extent of smoking history that are acknowledged and make it difficult to extract a simple single marker that indicates the quantity of
exposure to tobacco smoke for any individual. These include variables such as the number of cigarettes smoked per day and the number of years of smoking, with vagaries around numbers of cigarettes smoked during particular years as well as types and quantities of tobacco and other substances. Passive smoking is also a health risk known to be significant, but is even more difficult to quantify in a way that is accurate enough to be useful in a research context.

5.4.2.5.3  BMI and TBIs

The correlation between BMIs and TBIs across the total sample indicated no relationship between these two variables. This finding was unexpected, as an inverse relationship between BMIs and TBIs was predictable and has been described in other research (Gyawali et al., 2014). High BMI scores predominated. The lack of spread in data values for both BMIs and TBIs could account for this insignificant result.

5.4.2.5.4  Mobility and TBIs

Mobility was not significantly associated with the baseline TBIs. Mobility later emerged after intervention as the most powerful predictor of effect of GTN on TBIs. Prescription of exercise in conjunction with GTN may well enhance any effect of GTN.

5.4.2.6  Associations of medical conditions with TBIs

Medical conditions are known to affect the incidence and pathogenesis of PAD. Cardiovascular disease and diabetes are known for their particularly significant associations with PAD and thus, by inference, inverse relationships were expected between medical conditions and TBI values. All these participants had PAD by definition of low TBIs at baseline, 44% were exsmokers, with the mean for years of smoking high at 34 years. Seventy nine percent were hypertensive, 65% had hyperlipidaemia, and 87% were medicated for other conditions. For this sample, some surprising absences in expected associations emerged. These are discussed in the subsections that follow.

5.4.2.6.1  Diabetes and TBIs
In people with diabetes, higher TBIs were associated with the greater number of years of diabetes, for the HbTBI foot only. This was contrary to expectations, as lower TBIs with both feet was considered the most likely finding with increased duration of diabetes.

The number of possible false positives from calcified vessels in those with long-term diabetes is unknown but needs to be considered particularly in light of these results.

There were no significant differences found in TBIs in relation to HbA1c for less than or greater than 10 years with diabetes, or for those with type 1 versus type 2 diabetes, or for those taking insulin. This sample had HbA1c values that indicated good diabetic control compared with other populations with diabetes described in the literature (Saydah et al., 2004), which may have accounted to some degree for the lack of association found between their diabetes and TBIs.

5.4.2.6.2  Hypertension, and TBIs

In this study, there was no association between use of antihypertensive medication and TBIs. From all analyses, the results were consistent and insignificant. Further study is needed to determine any relationship between hypertension, antihypertensive use, and TBIs.

Managed hypertension is likely to lower overall blood pressure effectively. This is a potentially confounding variable that would be at play in all participants taking antihypertensive medication. Whether this medication also lowers TBIs, or whether the effects of managing hypertension would maintain or increase TBIs, requires specific pharmacological study.

5.4.2.6.3  Hyperlipidaemia and TBIs

No significant relationship emerged between hyperlipidaemia and TBIs. Hyperlipidaemia is known to be associated with atherosclerosis, and therefore lower TBIs from impairment in supply vessels was expected. This result suggests that, similarly to the antihypertensives, those on hypolipidaemic medication and under medical management for these cardiovascular risk factors would have this controlling element in place which is probably protective against low TBIs from atheroma, depending on the effectiveness of the
intervention and the compliance of individuals with their prescribed medication regimens. Further study on the extent of disease is needed to determine any relationship between TBI and hyperlipidaemia.

5.4.3 Results concerning GTN

In terms of raw data, all groups except the control group increased in their TBIs of the LbTBI foot during the course of the study. Increases on the HbTBI foot were also present but much smaller. There is a danger, however, in looking at raw scores and more sophisticated analyses such as ANCOVA are more valid.

5.4.3.1 Effect of GTN on TBIs

The effect of GTN on TBIs was positive, and this supports the primary hypothesis of the study demonstrating a treatment effect from GTN on increased perfusion. The fact that TBIs increased significantly with the use of GTN in both the high and low dose intervention groups compared with the control group, despite their greater related pathology and PAD risk factors, speaks even more strongly for the effect of GTN than is suggested by a cursory evaluation of the final overall results.

The significant effect of the larger dose at Visit 3 compared with the control group (as seen in the ANCOVA presented in Table 5.38) has clinical relevance for the application of GTN to the problems of pedal ischaemia. At this same early timepoint, the lower dose did not produce a significant increase in TBIs. The high dose produced higher TBIs versus the low dose, and the difference at Visit 3 was significant.

These results point to the higher 2.5 mg dose being an effective and clinically valuable dose one month after the intervention. The effect for the high dose was greatest at one month, and was still significant but reduced by 5 months.

By Visit 7, (which was 5 months after commencement of the intervention at Visit 2), there were significant effects for both doses as shown in the ANCOVA in Table 5.42. Although the lower dose was much slower to show an effect, it produced overall the greater change from a lower baseline start position. This indicates that both the low dose and the
high dose were significantly associated with increases in TBI and this therefore suggests that both doses have clinical value.

Effectiveness of a 2.5 mg dose was also determined by Wei et al. (2011) from their meta-analysis of nitrates for use in stable angina. They concluded that constant use of the 2.5 mg dose, which was below the tolerance threshold, was more effective than were the standard doses that were 12 times higher but had to be used intermittently to avoid tolerance. The confirmation of the effectiveness of these two low doses on PAD for pedal ischaemia is the main outcome of this enquiry and indicates that it may be of clinical value for wound healing applications. If doses can be kept below the tolerance threshold and thereby provide 24 hour effects with continuous use, this could be most useful for wound healing and potentially also for wound prophylaxis in periods of risk of breakdown. Longer term effects may be obtained with the low dose. A regimen that may prove of the greatest value could commence with a 2.5 mg dose, then drop to a 1.25 dose. Further research is needed to confirm this effect.

Doses were low by design as an aim of the study was to find the smallest dose that would effectively increase TBIs despite the known difficulties with large variation in absorption rates. Greater effects are likely to be found with larger doses, especially in people with PAD who are expected to have impaired absorption. These absorption impairments are probably associated with higher tolerance thresholds, but this is unknown for individuals until the effects are evident from monitored dose titration.

5.4.3.2 Effect of GTN: Timing of the first measure

The first month of the intervention between Visits 2 and 3 was very likely to have been a lost opportunity for monitoring the earliest of the GTN effects. The early pilot results from the case studies of Chapter 2 showed rapid ulcer healing and increased TPs. The longest time period for the 6-year-old ulcer to heal was 6 weeks. All other healing outcomes took place in much shorter periods from a minimum of 3 days. This timeframe is in contrast to the first vascular measurements of the experiment reported within this chapter, namely 4 weeks after the intervention. At this timepoint, the maximum TBI increase of an intervention group at any timepoint in the study was evident, being a significant increase on the high dose foot relative to the control. This raises the question whether even greater
effects may have been occurring in the earliest weeks that escaped detection with this schedule of monthly monitoring. Tolerance effects may have eventually come into play and attenuated later effects.

The meta-analysis of Wei et al. (2011) combined data from 51 studies. The timespans were usually short, with 26 of these trials lasting less than a week, 18 lasting between 1 and 2 weeks, and the longest trials being 6, 8, and 12 weeks. These timeframes were in contrast with the 5-month span of this enquiry.

5.4.3.3 General vs local effect

The effect of increased perfusion from GTN at the doses studied was only a unilateral one in 75% of the 12 subjects who had a headache response. By the time the first follow-up TBI measures were taken 1 month after the intervention, 75% of the people who had initially experienced headaches had increases in TBIs only on the treated foot. This strongly suggests that a local effect is more likely than is a general effect with the GTN doses used in this study. Because headaches occurred with the greatest frequency within two days after patch application, the temporal details of this general versus local effect were undetectable given the monthly measurement schedule.

A need for localised vasodilation in peripheral ischaemia has been identified by Francis, Hubbard, and Johnston (1977). As in the experiment reported in this chapter, they used topical treatment with GTN on one foot only and compared measurements taken from both feet. Their results also indicated that topical GTN effects were most likely to be localised. A local effect indicates clinical applicability for the treatment of peripheral ischaemia.

Even when generalised vasodilation is sustained with concomitant use of systemic antihypertensives, as was the case in the majority (79%) of the subjects in the present experiment, additional local peripheral vasodilation was associated with GTN use. The use of antihypertensives was similar across intervention and nonintervention groups in this study when both intervention groups and both control groups were combined. However, the largest number of participants taking antihypertensives was in the high dose group at 91.7% and the lowest was in the control group at 61.9%. The difference between these two groups
was significant \( (p = .029) \). Particularly because the ANCOVA indicated that the high dose group had the most significant initial effect from GTN, it may be concluded that GTN is effective for increasing local peripheral vasodilation even in the presence of systemic antihypertensive medication.

**5.4.3.4 Responders and nonresponders to GTN**

The relationships between GTN outcomes at Visit 7 and health and demographic variables made up the final section of the results (Section 5.3.8), and were summarised in Table 5.44. Three significant differences between the responders to GTN and nonresponders emerged under analysis in the domains of diabetes, smoking for more than 10 years, and mobility.

People with diabetes were significantly less likely to respond to GTN. This result needs to be taken into account to inform expectations of outcomes for people with diabetes. The proportion of responders and nonresponders in this study suggests that the number needed to treat for an effect with diabetes is 2–5. Greater effects may be seen at earlier timepoints with shorter term exposures and using individually titrated doses.

Smoking for more than 10 years was associated with a significantly poorer response to GTN. The proportion of responders and nonresponders in the study suggests that in long-term smokers, the number needed to treat for a response to GTN is 4–5.

These numbers needed to treat were derived from this sample where the low dose was taken by half the intervention group. Given the results that have emerged favouring the effect of the larger dose over the low dose for the short-term effect, further research based on people with diabetes and a history of long-term smoking, i.e., people who are at greatest risk of ischaemic foot pathology, to more accurately assess the value of GTN would be warranted. This research should focus on individually titrated dosing regimens starting at 2.5 mg to maximise results with short-term frequent measurements.

Discretionary mobility was by far the most significant predictor of effectiveness of GTN (see Table 5.44). The two mobility groups in the study were distinguished by participating in discretionary recreational activity versus those performing only activities of daily living.
without extra recreational activity. In the lower activity group there was only one GTN responder and there were 8 nonresponders. In the more active group there were 15 nonresponders and 22 responders. This gives rise to the question whether increased mobility created a condition more conducive to a response to GTN, or whether the better health of people who were more active was associated with greater likelihood of a GTN response.

5.4.3.5 Unexpected placebo outcomes

The placebo group matched the high GTN dose group with an initial average increase of 0.11 from baseline to Visit 3, and then remained steady at Visit 7.

The greatest contrast is the comparison between the placebo and control group. This unexpected result casts doubt on the results overall and raises questions about the study, including the homogeneity of the groups and the possibility of the placebo effect having significant influence.

The findings for the placebo group invite enquiry regarding whether the effect of GTN is similar to a placebo or whether the performance of the placebo group was merely a chance finding.

A reported phenomenon is noted where the enthusiasm of the researcher combined with the novelty of the intervention is associated with higher placebo responses (M. Nott, personal communication, 7 October 2014). This phenomenon could apply to this study and to myself as the sole data collector.

Authors investigating side effects with transdermal GTN in a controlled trial (Paolini, Appleyard, Nelson, & Murrell, 2005) describe a result demonstrating the prevalence and suggestibility of headaches. Both the placebo group and the active intervention group were informed of possible headaches. Of the placebo recipients, 33% reported headaches, taking the supplied pain relief for headache at a rate of a mean of 8 tablets per subject over 6 months. In the GTN treatment group, 66% reported headaches and this group took pain relief at a rate of 9 tablets per subject. These results underline the prevalence and
suggestibility of headache, and the subjective nature of research, even in areas of enquiry where the outcomes might be assumed to be mainly physiological.

Antigoagulant use (almost exclusively aspirin, with its anticoagulant action of platelet inhibition) was spread unevenly across the four groups with the largest difference between the low GTN group at 51% and the placebo group at 68%. Chi square analysis indicated that this difference was not statistically significant. However, the placebo group had the greater percentage of anticoagulant, and therefore aspirin, takers. The effect of aspirin on TBIs is currently unknown but could have a protective effect in its improvement of capillarity by making red blood cells more flexible and therefore the vascular supply more able to perfuse the smallest terminal vessels. Small vessel lumen with attenuated vasodilatory ability is prevalent in PAD.

The Hawthorne effect describes the phenomenon of generalised improvements seen as a result of being involved in a study. The Hawthorne effect might also contribute to the placebo effect and that group’s results. Perhaps the act of the daily patch application in all three of the patch groups compared with the control enhanced footcare awareness and health behaviours in ways that influenced the outcomes. Some of these mechanisms might have been related to self-care practices such as regularity of taking other medications; enhanced awareness of the feet, footwear and foot care; and, by possible extrapolation, perhaps practices such as healthier dietary and exercise behaviours.

5.4.4 Study limitations

A number of limitations associated with this experiment have already been acknowledged and discussed. These included the compromise of the blind randomization process originally intended, the timing of measurements missing the short term effects, which were potentially the most powerful, the small and low dose range used, and the data collected that was not suitable to bring forward for further analyses.

The tests used in the main study had all been previously validated in other contexts as presented in the literature review, and were not evaluated for reliability with the single data collector. This may have been a source of some error inherent in the trial.
An additional limitation was the taking of BPs on one arm only when best practice should have included both arms and the use of the highest systolic pressure in all TBI calculations. This approach, using only the left arm systolic BPs, would have missed potentially important IADs in this cohort and could have been used to relate IADs with interfoot differences. The use of the higher of the two brachial BPs would have given a better representation of the systemic BP supplying the rest of the body, thus increasing the accuracy of calculations of TBIs. Systolic BPs may have been attenuated by stenosis in either arm as is reportedly present in 5% of people with hypertension (Clarke et al., 2012).

All three patch groups were made aware of the possibility of side effects, and some additional verbal information was given about possible headaches to those in the intervention groups. This same additional verbal detail was not given to placebo group participants. This should have been made consistent across all patch groups with standardised information.

One of the primary original intentions of the study was to be able to answer questions about GTN and ulcer healing as described in the third hypothesis. As described earlier, this was not achieved due to the population not being specifically recruited for wounds. To answer this question, future studies specifically seeking people with wounds should make this a focus of recruitment and study design.

A larger sample size would have been useful to confirm and clarify the findings of this study and could have included more of the topics originally envisaged and delineated in the three hypotheses.

5.4.5 Summary

Several findings of this chapter regarding TBI measurements add to the body of knowledge in this area. They confirm the principle that single TBI readings are not best practice, and point to the need for appropriate data management in future TBI research. The importance of serial measures to enhance the validity of conclusions is underscored by understanding the fluctuant nature of TBIs, especially in the presence of relevant pathology. Specific
pathology such as cardiac output irregularities and respiratory disorders were probably associated with high levels of TBI fluctuations in this sample.

Although the study groups were well matched for cardiovascular pathology and cardiovascular medications, the intervention group members had more years of smoking history, took more medications and had more medical conditions. (Only the latter difference reached significance in the comparison of nonintervention to intervention group pairs). Therefore the positive effect found in the GTN treatment groups is even stronger, with many of their members having overcome these relative health disadvantages.

Some of the results from this experiment are not as clear-cut as hoped or anticipated. They provide only partial support for the hypotheses that the doses of GTN used in this research would be associated with increased TBIs. The strong performance of the placebo group casts questions over the meaning of the results as a whole and could arguably disconfirm the hypothesis that GTN can raise TBIs. However, the high GTN group had significantly higher TBIs than did the control group at Visit 3, and both the low and high groups had significantly higher TBIs relative to the control group at Visit 7. The significant differences that occurred for both doses of GTN warrant further research. The following chapter is dedicated to a more detailed and comprehensive discussion of the broader implications of these results. Areas recommended for follow up and related research will also be discussed there.
6.1 Introduction

In this chapter, integration of the outcomes of this work is provided with a focus on implications for clinical practice and on elements that are valuable for future research. Consensus about the use of TBI versus ABI is not universal. Some of these arguments are presented, with illumination of related issues and sources of contention.

A novel vascular assessment protocol has been developed using the evidence-based tests incorporating TBIs and other elements with the highest sensitivity and specificity. Throughout this chapter, recommendations about suitable areas for further study on both toe pressures and GTN are identified. A set of recommendations for procedures for the use of GTN in peripheral arterial disease (PAD) is provided. Finally, a summary concludes this thesis with a concentration on its key findings.

6.2 Implications for podiatry education

This work revealed some implications for podiatry education at an undergraduate level. The literature indicates that testing is subject to reduced accuracy with inexperienced examiners for vibration with tuning fork and for pulse palpation. Results of the pilot study highlighted questionable validity for vibration and temperature discrimination. The vagaries of students taking a pain history were also exposed. Neurovascular testing contributes to accurate clinical decision making only when it is accurate and valid. Focus on the appropriate choice of tests, the methods by which they are administered and the soundness of the evidence they add to the overall examination are worthy of critical evaluation. From this research, it is clear that each of these elements should be scrutinized for validity and suitability, and education in these areas should be updated to reflect the latest body of evidence.
6.3 TBIs and vascular assessment

6.3.1 Variability of TBIs

Artefacts known to cause blood pressure increases are associated with all blood pressure measurements, including those of the periphery. These are as presented in Table 4.2 and covered in the experiment-specific discussion in Chapter 5. The variable peripheral resistance at the end of an arterial supply line due to capillary exchange pressure is also implicated. The local effects of thermoregulation and local peripheral endothelial reactions are very significant factors affecting toe pressures. These physiological homeostatic functions are known to be variously impaired in diabetes. As well as these intrinsic causes of TBI variation, the items regarding test protocols in Appendix C influence measurement outcomes and can easily invalidate the results.

6.3.2 Incorporating toe pressures in evidence-based vascular assessment

The background to the development of an assessment guide is offered here with some refreshment of the relevant context linked to this thesis. PAD is asymptomatic in 50% of cases (Nogren et al., 2007) and therefore people with this condition are often undiagnosed. The palpation of pedal pulses is not sensitive or specific in PAD in the presence of diabetes and even less so with concomitant neuropathy (McGee & Boyko, 1998; Xu et al., 2010). Biphasic Doppler sounds and tracings can be present in both normal and impaired vascular supply states (Williams et al., 2005). The confirmation of a monophasic Doppler signal is a highly sensitive and reliable indicator of PAD (Williams et al., 2005). However, biphasic Doppler sounds are the most common finding and are routinely and mistakenly used in practice as indicators to discontinue vascular assessment in the mistaken assumption that they indicate reasonable supply that is age-appropriately reduced from the normal triphasic supply typical of a younger population. The majority of people in the study that is the subject of this thesis had biphasic pulses despite their impaired TBIs.

In order to overcome the problem posed by Høyer et al. (2013b), whereby current methods of assessment fail to identify around 30%–60% of people with PAD, an original alternative process has been developed and refined into a “clinician-friendly” flowchart. This flowchart, presented in figure 6.1 with another more annotated version in Appendix L, provides the structure for an evidence-based vascular assessment process. This process
avoids errors inherent in traditional assessment from the under-appreciation of the low sensitivity and specificity of commonly used assessments such as visual vascular signs and ABI in at-risk groups. This flowchart comprises contemporary evidence pertaining to vascular assessment and provides a practical framework for clinicians to use in lower limb vascular assessment. One of its major purposes is to enable new or prospective users of TBIIs to integrate them appropriately into their vascular assessments. This assessment pathway enables determination and monitoring of peripheral arterial status to inform clinical decision making and appropriate and targeted medical referrals.

In order to conduct a valid and evidence-based vascular assessment of the feet, some main points need to be incorporated in the process. (See Figure 6.1 for the flowchart incorporating these tests.) These are:

- Diabetes, renal disease, and advanced age render ABI an insensitive test.
- In contrast to other visual signs, Buerger’s sign is 100% specific for severe PAD.
- The appropriate use of ABIs vs TBIIs is indicated by the medical history and Doppler findings.
- End outcomes from this assessment include, as well as monitoring and referring for PAD itself, the significantly increased CVD risks identified by IADs in blood pressure, and by the presence of PAD.

In addition to the risks of PAD to foot health, mobility, and quality of life, PAD carries an 85% increased risk of cardiovascular mortality from CVA and myocardial infarction. Because of this risk, health professionals performing vascular assessments are in a position of significant professional responsibility to undertake screening for previously undiagnosed cases of PAD and to refer at-risk patients for optimal medical management. This has not previously been incorporated into podiatric practice but is highlighted here as a professional responsibility in the wider context of the holistic health of the test subject. The comparative sensitivity and specificity of this approach in the diagnosis of PAD and identification of CVD risk should be further evaluated with appropriate research.
The flowchart following in figure 6.1 is provided as a guide to evidence-based vascular assessment that incorporates vascular tests with acceptable sensitivity and specificity in order to enable appropriate diagnosis, referral, and stratification of risk status for peripheral artery disease (PAD) and cardiovascular disease (CVD). Monitoring recommendations are determined from the risk status.

Figure 6.1 Vascular Assessment Flowchart
1. **History**- Diabetes, neuropathy, advanced age, kidney disease are linked with vascular disease and reduced sensitivity of tests.

2. **Visual signs**
   Buerger's sign is the only visual sign that is sensitive for severe PAD.

3. **Palpation**
   Pulses have low sensitivity and specificity for PAD in vascular disease.

4. **Doppler sounds and waveforms**
   Doppler ultrasound is highly sensitive and specific for PAD.

   - **Monophasic:** Abnormal
   - **Biphasic:** Ambiguous
   - **Triphasic:** Normal

5. **ABI/TBI**
   Choose TBI over ABI in diabetes, aged, or in vascular conditions.
   - ABI is abnormal if < 0.8 or > 1.2. Normal range ABI is ambiguous in vascular disease.
   - Absolute toe and ankle pressures may be more reliable than indices in severe vascular disease.
   - TBI < 0.65 indicates PAD
   - > 0.71 excludes PAD
   - Use 2.5cm cuff size as the default.
   - Screen for inter arm systolic differences and refer when >10 mmHg.

   - **Abnormal result:**
     Repeat measurement + medical referral + routine reassessments
   - **Borderline results:**
     Repeat measurements + medical report + routine reassessments
   - **Normal results:**
     Routine reassessment:
     Annual vascular review if over 65 or over 50 with other risk factors.
6.3.3 TBI protocol issues

Further study of TBI test protocol details such as pre-test rest times, cuff size effects, effects of repeated measures, and timing between tests to best manage the effects of vascular hyperaemia might add considerably to the reproducibility and validity of test results.

Cuff size is not usually mentioned in reports of toe pressure research and can be assumed therefore to be not appreciated or usually considered. Clinicians and researchers unaware of this difference may always choose the small occlusion cuff because it can always be fitted on the toe along with the additional sensor cuff. Another pitfall is to use the two sizes indiscriminately, not understanding the differences that this makes to the readings. Cuff sizes should be the subject of further research and should be taken into account and be reported along with pressure measurements in all studies as well as in clinical practice.

6.3.4 Significance of differences of TBIs between feet

The differences in TBI between the two feet of each participant at baseline reached a high level of significance \( p < .001 \) as reported in Section 5.3.4.4. Significant interfoot differences in people with PAD might be common and may be correlated with the degree of vascular pathology. Interfoot differences might also offer a link to cardiovascular risk status as described by Clarke et al. (2014) for IADs. Insufficient literature currently exists to answer the question whether significant differences indicate pathology or are a normal occurrence.

6.3.5 Vascular hyperaemia

The phenomenon of a reactive vascular hyperaemia in which a flush of increased vascular perfusion follows a period of constriction (such as from a tourniquet or a pressure cuff), is well documented and was therefore expected to be present and require management in the context of this enquiry. However, after analysis of the three TBIs taken at each episode, it was clear that because the first measure was not significantly different from the other two measures, it did not apply as a relevant issue in the sample selected for this study. This raises the question of whether vascular hyperaemia is indeed present in a cohort with PAD,
or whether it is a feature only of more normal or perhaps unimpaired peripheral vascular circulation.

### 6.3.6 Manual versus automated toe pressure systems

A controlled experiment to compare the readings obtained with manual versus automated TBI measurement systems would be valuable. The reports of the users of automated systems compared with laboratory measures are positive (Bonham et al., 2010; Harrison, Lin, Blakely, & Tanaka, 2011; Høyer et al., 2013b; Perez-Martin et al., 2010) reporting on the accuracy of this novel technology compared with laboratory testing. However, there appears to be no published direct comparison of the two clinical manual and automatic system types. The manual systems are quick and easy to use, and although the calculations need to be performed manually, the mechanisms are easier to troubleshoot in this transparent and simple method. However, their reliability is questionable (Romanos et al., 2010). It would be useful to have comparisons of manual and automated systems with strict adherence to the same test protocol.

Currently available systems include the French Systoe, the Japanese Vasera, the Canadian Koven, the American Hokansen, and the British Hadeco system. The PeriPlus 5000, a Swedish system, has a unique feature to address the issue of consistent warming with an inbuilt toe warming element around the sensor. Controlled comparisons between these devices would provide useful information for clinicians and researchers in this field, leading to consensus and determinations of validity to assist further investigation.

### 6.3.7 Toe pressure and temperature

Sawka and Carter (1992) report significant temperature effects of toe pressure measurements taken between 10 °C compared with 30 °C, noting that pressures increase with temperatures and that cold temperatures reduce pressures and create an unreliable test environment.

Future researchers should remain vigilant for temperature effects and ensure that measures are taken within the small range of recommended ambient temperatures. It could be that the controlled temperature environments in which the study was conducted
regulated any effects of climatic temperature variables on the skin temperature of participants.

6.4. GTN issues

6.4.1 Temperature effects

As described in the lit review, as well as the documented floor on measurable toe pressures in cold conditions when skin temperatures are < 20 °C, conversely, warm conditions are known to enhance vascular supply to pedal extremities. GTN is known to have markedly increased effects when skin is well perfused, and both skin and ambient temperatures have been linked with significantly increased absorption (Barkve, Langseth-Manrique, Bredesen, & Gjesdal, 1986; Coakley, 1983; Klemsdal, Gjesdal, & Bredesen, 1992). Future researchers should take advantage of this knowledge and ensure that the feet stay as warm as possible to maximise the absorption of GTN. This fivefold increase with 20 minutes of heat exposure is a potentially powerful way to enhance GTN effects. The use of warm environments, and extrinsic sources of warmth such as electrically powered footwarmers, might be considered for people in conjunction with GTN treatment.

Although insufficient numbers of participants were involved in this intervention to assess the side issue of effects of warm socks and footwear, there were several occasions when participants commenced wearing a specifically recommended brand of markedly more effective insulating socks, and sometimes shoes. This recommendation was given to some study participants when their feet showed effects of cold exposure or participants complained of painfully cold feet. Large increases in toe pressure were noted on the handful of occasions when this occurred, leading to interest in the potential clinical significance and quantifiable effectiveness of this simple, drug free intervention. A clinically useful piece of research would be to test for the contrast in GTN effect with and without warm socks and insulating footwear.

Exploration of these reported links between temperatures of skin, test environment, and GTN absorption, particularly in feet with PAD, would shed light on the importance of maintaining the temperature of the feet for optimal vascular perfusion.
6.4.2 Exercise effects

The strongest predictor of GTN effect in this study was the distinction between the groups who performed some discretionary exercise. It would be valuable to determine how much of the improvement in effect is due to the exercise itself, and how much is due to warming as a secondary outcome of exercise. If warming alone is valuable, it could be applied when people are unable to exercise. It may also mean that people with less pathology who are therefore more active, are much more likely to experience a positive effect from GTN.

In the same study in which GTN absorption was increased fivefold from a sauna (Barkve et al., 1983), participants were tested for GTN patch absorption on another day when a bicycle ergometer ride was performed. Mean GTN plasma concentration increased relative to the control condition by 2–3 fold with this exercise.

A relatively lower degree of pathology can be broadly inferred from mobility differences given the association with physical function, particularly walking speed and longevity (Cesari et al., 2006). The very strong links between wellness and mobility are irrefutable. If GTN is absorbed more readily with exercise, this could lead to enhancement of its effect by a GTN prescription combined with a concomitant exercise recommendation in suitable cases.

6.4.3 Dose and GTN regimen questions

There is difficulty inherent in determining the most useful dose for any individual. This problem is two-pronged due to the dose ceiling over which tolerance quickly occurs and the lower end range were effectiveness might lie, but both of these high and low endpoints are difficult to establish due to wide-ranging individual differences in absorption up to tenfold. The effect of local vasodilation to the feet has not been quantifiable before toe pressures became accessible as clinical measures. An additional potential problem exists, highlighted by the work of Rizk, Witte, and Barbul (2004), in which although nitric oxide supplementation was helpful to address nitric oxide (NO) deficits in wound healing, very high levels of NO were found to impair healing. These researchers state that more work is needed to clarify this point, but there are animal wound healing studies in which improvements occurred, including angiogenesis, faster wound closure, and greater collagen
organisation when using NO donors, as previously noted in the literature review, Chapter 3 (Rizk, Witte, & Barbul, 2004).

The Level II evidence regarding dose determination for tendinopathy (Paolini et al., 2006) is the most detailed work on the topic of dose determination in the periphery. However, with the large individual variations reported in absorption, compounded by the difficulties of reduced perfusion in the periphery in cases of PAD, individual dose titration to effect seems to be advisable to overcome these issues.

The titration process reported by Rayman, Baker, and Krishnan (2003) for painful diabetic neuropathy uses a start-up dose of 5 mg on one foot, and if side effects occur, reduces this to 2.5 mg. If that dose is tolerated, the dose is increased to 5 mg on each foot. If side effects are problematic, they suggest splitting the original dose using the 2.5 mg dose on each foot only. They describe an effectiveness of 44% using this regimen.

Pulsed therapy is recommended for some drugs that cause tolerance or cumulative likelihood of unwanted side effects. If GTN is proved to be most effective in the short term, as seems possible from the results of this study, short- to medium-term pulsed dose regimens may be a useful alternative way to apply this therapy.

In the main experiment of this thesis, the results at 5 months for the low dose represented the greatest increase of the two doses in TBIs from baseline to the end point. As suggested in the previous chapter, following on from the strength of the effects seen from the two different doses as shown in Figures 5.9 and 5.10, the regimen that may prove to be of greatest value could commence with a 2.5 mg dose for the most powerful short-term effect over the first month, then drop to a 1.25 dose for the strongest maintenance effect. Further research is needed to confirm the different effects of doses in relation to time.

### 6.4.4 Local versus systemic effects

The results of the main thesis experiment indicated that the effect of GTN was by far more likely to be local rather than general. This is useful in supporting the clinical value of this application of GTN for the problems associated with relative ischemia of the periphery.
where local vasodilation is desirable, but general effects of hypotension and headache are undesirable.

Confirming this local effect, Rayman et al. (2003) stated that in their experiment using patches for painful diabetic neuropathy (PDN), when only one patch was used, most patients reported a reduction in pain solely in the leg to which the patch was applied. This also suggests a local mechanism rather than a systemic effect.

### 6.4.5 Timing of testing, looking for shorter term effects

The ideal timing of the greatest effects from GTN was unfortunately not captured given the design of this experiment. The rationale for choosing monthly monitoring was to give time for any angiogenesis to occur and for a maximal effect to become apparent over 5 months as a medium term if indeed this effect was present. Future research should focus on shorter timespans directly after the initial application with measurements repeated regularly, even daily, to establish the true extent of short-term effects. Adding support to this theory of powerful short-term effects is the headache that occurred in 25% of active drug participants largely during the first 2 days. Measurement of toe pressures during this time and over the first 3 weeks of use would be an area that is most likely to produce useful results regarding the maximum potential of GTN to increase TBIs in PAD in the short term.

From the pattern of headaches predominating in the main study of this thesis, the effect of vasodilation from GTN could be assumed to be a short-term, generalised effect, but in the short- to medium-term, it seems after the first two days, the effect changes primarily to a local one for most people. This concept needs to be further understood and integrated in its application for problems of pedal ischemia.

The doses used in the four case studies described in Chapter 2 were equal to or greater than those used in the main experiment. Doses of the case studies ranged between 2.5 and 5 mg. This dose range lies within that used by Rayman et al. (2003), namely 2.5–10 mg. The higher end doses are likely to be revealed with further study to be more useful than the lower doses used in this experiment (1.25 and 2.5 mg). The rationale for the choice of this dose range was based on relevant literature and is given at the start of Chapter 5. A conservative approach was taken, using the margin for greatest safety given the novelty of the application of this drug on feet in an ischaemic population. Higher dose ranges would
probably be more useful in this cohort where advanced PAD is responsible for reduced perfusion and therefore absorption. Researchers should explore the relationship of doses of 1.25, 2.5, 5 mg, and higher for their effectiveness in PAD over time. Titrating up until headache occurs may be useful to determine whether absorption of adequate levels has occurred. If the headache is mild, temporary, and only of short-term duration, this could function as a useful clinical indicator to help determine individuals’ doses.

Research on noncontinuous dosing regimens should be explored in future investigation of GTN as a treatment in PAD to determine effectiveness and whether tolerance is an issue for populations with PAD.

6.4.6 Differences in GTN absorption

One difficulty in determination an effective dose is the phenomenon of tenfold individual differences in absorption of GTN. The factors of cardiac output and existing skin perfusion are known to play a role (Klemsdahl et al., 1992). However, much of this issue remains not well understood. Absorption variation has a large bearing on determining the correct dose, particularly in determining any modifiable factors that contribute. This is one of the greatest challenges involved in determination of effective application of GTN in pedal ischaemia.

Haebisch (1995), studying individual differences in the effect of GTN, found that greater GTN absorption (associated with increased skin temperature) was positively correlated with higher skin fold thickness and body fat content, being greater in obese than lean people and that obese women had the greatest absorption. The GTN patches in this experiment were placed on the forearms of participants. The typically different fat distribution in men and women, with more central adiposity typical in males and generalised adiposity in women may account for the increased absorption seen in women relative to men in this study.
6.4.7 Clinical pathway for individualised GTN regimens

As presented in the literature review, titrating the dose of GTN for individual effectiveness is recommended and is described by Rayman, Baker, and Krishnan (2003). Ten fold differences in GTN absorption and effects, as reported by Gjesdal et al. (1985) and by Francis, Hubbard, and Johnston (1977) underscore the need for individualised treatment. In Appendix M, a set of guidelines for the use of GTN by prescribers is provided. The recommendations within these guidelines aim to provide a pathway to negotiate the issues of dose determination associated with using GTN for PAD specific for an individual presenting to their doctor in a clinical situation. The contraindications and problems with variable absorption and tolerance are outlined in a concise form.

The information guide for use of GTN in Appendix M lays out a plan for treatment including titration from a start-up dose of 2.5 mg. This guide was developed for the medical practitioner prescribing the GTN and is supported by client information (in Appendix N) that is associated with a chart (Appendix O) to track doses and their associated effects to assist in dose titration. This chart is for use by the patient and is the main tool to assist the prescribing practitioner to choose the most appropriate individual dose. The desired effects as well as any side effects from the drug are recorded by the patient. This document is designed to take advantage of the phenomenon of headache that commonly occurs with GTN use to indicate that absorption has taken place.

This guide and the use of the tracking chart should be subject to testing to determine the validity and associated practicalities of this approach. This issue should be the topic of further inquiry to determine the factors that can be anticipated and used to adjust the dose appropriately for individual effectiveness.

Although Wei et al. (2011) reviewed 53 studies in which they determined that doses below 2.5 mg do not result in tolerance, these studies used application of the patches to the chest for management of angina pectoris. Different sites of application could provide variable absorption due to differences in skin such as epidermal and dermal thickness, subcutaneous fat, and vascularity of underlying structures. The most important factors affecting the issue of absorption might be assumed to be the skin permeability and vascular
perfusion at the application site, with adiposity being an important variable due to potentially higher relative vascularity compared with lean tissue.

The work of Gjesdal et al. (1985) showed marked differences in GTN in plasma levels of blood taken from different anatomical locations. In their study on gunpowder manufacturing workers, blood from cubital (wrist) vessels had nitrate levels that were up to 100 times higher than had blood taken from femoral (thigh) vessels of the same subject at the same time. This issue is essential for incorporation into research if accurate determination of absorption is planned for analysis by blood sampling. It also is another piece of evidence supporting the phenomenon of a localisation of effect closest to the site of exposure.

6.4.8 Headache as an indicator of systemic absorption aiding dose titration

In search of a useful indicator for the absorption of an effective dose, the commonly associated phenomenon of headache may be valuable, although only 25% of participants on the active treatment had headaches at these low doses. Side effects are dose dependent. The headache produced by the generalised vasodilatory effect in the study was usually mild and short term. See Chapter 5 Section 5.3.7 with regard to side effects and local versus generalised effects in the results of the experiment. In the meta-analysis by Wei et al. (2011) on a variety of doses of GTN for angina, headache was reported in 51.6% of study participants.

Although headache was not usually associated with vasodilation on the opposite foot to the treatment side, its presence nevertheless indicated that a short-term general effect was taking place if only for the first two days when headaches affected 25% of those in the intervention groups. This area of investigation could lead to clearer dose titration guidelines and determination of individually tailored doses. Studying this primarily in normal populations, without the potential contralateral differences that seem to be common in PAD, would be a logical approach for the next stage of research in this area.

6.4.9 Safety issues

6.4.9.1 Vascular shunting
Shunting is a concern that has been discussed by some authors in regard to the therapy of peripheral vasodilation in the case of digital ischemia, notably by Hubbard, Francis, and Johnson (1977). In systemic vasodilator therapy, the vascular supply is diverted from the most damaged and least responsive vessels by generalised vasodilation which is most effective in the least diseased vessels. The theory is that this relative increase in volume of the less diseased vessels can cause shunting away from the most ischemic parts. This is discussed as a precaution in the use of GTN in the chest in cases of COAD (MIMS Australia, 2015). This would depend on the proximity of the effects to the application site of the patch and the extent to which both local and generalised effects occur in the individual. Any vessel damage limiting vasodilation such as sclerosis or calcification would be likely to play a part in this effect.

Hubbard et al. (1977) cite other authors calling for the ideal agent that could selectively vasodilate the most ischemic areas. They also cite the American Medical Association’s Journal of New Drugs as saying in 1976 that the newly developed sustained release forms, (patches) probably would not enhance vasodilatory effect but would prolong pharmacological action.

This should be another area included in future research regarding GTN effects. It seems that the evidence that has been gathered by this study and the literature points to a very local effect in transdermal applications, which could indeed fill this therapeutic need for specifically targeted peripheral vasodilation.

6.4.9.2 Effects of GTN on venous incompetence

The effect of GTN is more profound on veins than on arteries, in terms of greater volume changes (MIMS Australia, 2015). De Berrazuetta et al. (1994) have successfully used GTN over the short term, reducing inflammation after sclerotherapy of varicose veins. The safety and value of GTN in cases of venous ulceration and incompetence is not otherwise reported in the literature.

If the benefits to healing listed from the literature review in Chapter 3 could be harnessed without detrimental venous pooling and stasis, GTN may find a place in the therapy for venous ulceration. However, the pathology associated with venous
incompetence caused by increased venous pressure suggests that GTN may be detrimental in these cases. This area should be investigated with careful controls in place such as effective compression therapy, and very close monitoring for any adverse effects.

6.4.9.3 Pilot case questions: Angiogenesis

From Chapter 2, regarding Case 3 who developed the marked telangiectasia, questions arise as to whether this represents a positive finding, representing superficial angiogenesis of the feet, or an increase of superficial vessels which is indicative of venous incompetence. This client continued to use GTN, delighted with the ongoing experiences of persistently warm and comfortable feet without lesions, which was in contrast to his experiences of many previous years. The cosmesis of the telangiectasia was of no concern to him and no other signs of venous incompetence were present. Further long-term monitoring of this and other cases would be useful to push out the boundaries of knowledge in this domain.

6.4.9.4 Safety of GTN in risk groups: diabetes, smokers

In Chapter 5 Section 5.3.8 the comparison of responders with nonresponders to GTN was made with some outcomes that might be valuable to pursue in future research. The presence of diabetes was among the three variables that were significantly related to lack of response to GTN. This could be viewed as disappointing in terms of the hopes for this treatment in people with diabetes. However, a close look at the figures of responders with diabetes reveals that it could be regarded as “a glass half full” or perhaps more accurately, one-third full. Of the people selected for their particularly good responses to GTN, about 1/3 had diabetes. People without diabetes were more likely to do well with GTN, and this was altogether expected given the known pathological associations.

Among the people selected from the active treatment groups for their markedly negative responses, 26% of the poor responders did not have diabetes while 74% of this group did have diabetes. Diabetes is significantly related to poor response, but 28% (10 out of 36) of the people with diabetes did respond well.

HbA1c, BMI, and years since diagnosis were all analysed but no significant results were obtained as to what characteristics distinguished the responders with diabetes from the
nonresponders with diabetes. This was counter to expectations and poses questions requiring larger and more diverse populations to determine any associations that may exist.

This research might be useful to direct therapy when it is most useful in those people with diabetes who are amenable to a therapeutic effect from GTN. The possibility that GTN was responsible for the other people with diabetes not responding should be considered also. The mechanism for this lack of effect might be the shunting described above from the most diseased vessels by the vasodilation of the healthier ones.

Smoking was another characteristic that produced significant differences in the comparison of responders and nonresponders. Any effect in the population of long-term smokers is worthy of further research as the cardio and peripheral vascular complications of these people are likely to make up a large part of the burden on the health care system.

Smoking history indicated that smokers in the control group had the lowest mean at 18 years of smoking, and this was significantly lower than the mean number of smoking years among smokers in the high and the low dose groups. Therefore, a comparison of treatment groups with control groups would not have been valid.

Questions arise with the outcomes of less response to GTN in people with a smoking history > 10 years. The most likely explanation for this result is that these people responded less because of the pathological effects of their smoking history on their vascular supply, but it is possible that this outcome was produced due to a deleterious effect from GTN such as shunting that lowered the TBIs. These questions should be considered and addressed in further research.

6.4.9.5 Preempting hot spot pathology

Future research on the effects of GTN for people who have diabetes and neuropathy should maintain awareness for the potential of the development of Charcot’s neuroparthropathy and hot spots indicating potential breakdown areas. Adherence to guidelines for temperature monitoring that are recommended as best practice for people at risk of Charcot’s neuroarthropathy and foot ulceration remains imperative in GTN research. With the addition of GTN, which may increase vascular supply and has not yet been
comprehensively studied in either normal or pathological groups, vigilance for any as-yet unreported deleterious inflammatory effects should be maintained by temperature monitoring.

6.4.10...Specific conditions

6.4.10.1 Antihypertensives for neuropathy

The various modes of action of most of the oral antihypertensives are largely associated with peripheral vasodilation. The question of how much additional benefit might be gained for local vasodilation with GTN patches on the feet for people taking already on systemic antihypertensives invites further specific pharmacological study. In this study, the addition of GTN did improve TBIs, and most notably in the high dose group in which 97% were taking antihypertensives.

The need for systemic antihypertensive management exists almost invariably in the population at high risk for foot problems due to underlying PAD. Generalised vasodilation is sustained with concomitant use of systemic antihypertensives, and these medications were taken by the majority of the study subjects. It is therefore important to explore the use of GTN in conjunction with antihypertensive medication.

The microvascular etiology of diabetic pathology is linked to potential treatments for diabetes and peripheral neuropathy. Harnessing the vasodilatory effects of two different types of antihypertensives in two separate studies, Malik et al. (1998), in an RCT on angiotensin converting enzyme (ACE) inhibitors, and Malik (2005), using angiotensin II receptor blockers, demonstrated that both of these approaches provide significant and meaningful improvements in vascular measures, such as vasodilatory responses blunted in diabetes, and also for neurological measures such as nerve conduction studies and symptom scores, with mild diabetic peripheral neuropathy in normotensive patients. These results could be considered an endorsement of the approach attempted with GTN in this study in both vascular and neurological parameters. However, using these systemic vasodilators in normotensive cases could cause hypotension. The recommendation of Malik (2005) is to use vasodilators with people in the early stages of disease to prevent endothelial damage as the use of antihypertensives is likely to be more valuable as a prophylactic measure than later in more advanced pathology.
Given the positive prophylactic effects on peripheral diabetic neuropathy with antihypertensive use, even in normotensive subjects with diabetes (Malik 2005; Malik et al., 1998), using GTN as a local vasodilator may provide this benefit and avoid problems of systemic hypotension. The additional value of local vasodilation for improvements in perfusion and the reported antineuropathic effects in this at-risk group with diabetes deserves more research attention.

Effects from antihypertensives would depend on individual differences and at what stage of the disease the hypertension management was commenced. The amount of vessel damage and sclerosis would make a difference to peripheral supply and to how effective any vessel dilator could be. Different types of antihypertensives have stronger effects on peripheral circulation, with calcium channel blockers best known for this effect. The thiazide diuretics would have less effect on the periphery due to their more central actions on kidney function.

6.4.10.2 Effects of GTN in neurological pain relief

For future studies on neurological pain relief with vasodilators including GTN, as with the Malik studies (Malik, 2005; Malik et al., 1998), it would be most suitable to recruit people with mid-range LOPS scores, indicating mild incipient neurological pathology that was the most amenable to reversal.

The body of literature existing for the effect of GTN on neurological pain relief is small but intriguing. With numbers needed to treat PDN with GTN reported to be 4–5 (Rayman et al., 2003) this is a treatment that would only help this fraction of sufferers and this effect may not reach statistical significance in a study. However, if GTN offers an alternative pain relief method, even in a proportion of people suffering from PDN, this would be of undisputed value for the individuals, and the flow-on effects for the community and the health systems could also be profound given the number of people being affected with diabetes in the worldwide pandemic of this disease. Determination of the patient types for whom this intervention is most likely to succeed would be worthwhile.
6.4.10.3 **GTN in intermittent claudication**

Intermittent claudication\(^1\) is an indication of severe PAD. In the experiment reported in Chapter 5, several participants in the active treatment groups with mobility limitation from intermittent claudication proffered unsolicited anecdotal reports of noticeable improvements in walking distances. Literature about the effect of sublingual GTN on improved walking distance reported an increase of 9% on flat ground and 19% walking on a treadmill (Walker, Heer, & MacSweeny, 1999). Intermittent claudication is a source of significant reduction in quality of life, which restricts mobility and reduces fitness. In a population where physical activity is vital to the optimal maintenance of cardiovascular function, any effect that enhances walking ability is worthy of exploration.

6.4.10.4 **GTN for Raynaud’s phenomenon**

There is a body of evidence that supports the use of topical GTN for people with Raynaud’s phenomenon, either primary or secondary to other conditions, for example, connective tissue disease or sclerosis (Anderson et al., 2002; Herrick, 2008, 2009; Herrick et al., 2011). The effects of Raynaud’s phenomenon in these people can be life affecting and result in pain, ulceration, and digital amputations. The evidence for the efficacy of topical GTN for this condition is unequivocal. Information about titration to determine the most effective dose and minimise side effects would be particularly helpful for this group.

6.4.11 **GTN supplementation**

The biochemical processes leading to the vasodilatory action of GTN hits a ceiling causing drug tolerance when thiol is depleted. It has been suggested that to aid in the associated biochemical processes, supplementation of GTN with alpha lipoic acid and vitamin C which can theoretically add thiol (Singh & Jialal, 2008) could be useful to minimise or overcome problems of GTN tolerance. Further exploration of the potential of this approach with research informed by relevant biochemical expertise may be warranted.

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\(^1\) Intermittent claudication is a condition in which diseased supply vessels lead to inadequate vascular supply for the demands of the calf muscle during exercise. Level walking is classically limited at a set distance that is much reduced with exertion and especially uphill terrain.
6.4.12 GTN and wound healing

As stated in the discussion section of Chapter 5, the third hypothesis regarding wound healing could not be confirmed by the findings in this study, but neither was it disconfirmed. Given the significant positive effects found on pedal perfusion in low doses in this high risk cohort, the load of pathology within the intervention groups, and the supportive associated literature—and notwithstanding the disconcerting placebo results—there is certainly a valid case for proceeding with further research in people with pedal wounds using low dose transdermal GTN. However, as has been acknowledged, GTN effects are most likely to be beneficial in cases with less advanced vascular pathology, and therefore may prove to be of greatest value in less chronic wounds and in wound prophylaxis.

6.5 Conclusion and summary of key points

The purpose of this thesis was to provide scientific insight to address pedal ischaemia, one of the main risk factors involved in high-risk foot pathology. This research enquiry was stimulated by the four case studies featuring rapidly healing foot ulcers. The pilot study examined the methodological practicalities by testing prospective assessment items including a novel toe pressure measuring device, and ensured that valid and reliable measures were in place to address the main research questions. From the subsequent experimental study of 100 people with pathologically low toe pressures, both the effectiveness of GTN in pedal ischaemia, and the TBIs of people with pedal ischaemia, were explored, resulting in positive and clinically useful outcomes.

The apparent effectiveness of GTN increasing TBIs when pedal ischaemia is present demonstrates that further research would be valuable. In the experiment, significant increases in TBIs occurred with the 2.5 mg dose at 1 month, and with doses of both 1.25 mg and 2.5 mg of GTN at 5 months, post intervention. The effects of increased vascular pressure were determined as being likely to be only local and to persist in the presence of systemic vasodilatory medication for hypertension. These findings have positive implications for the use of GTN in target populations with pedal ischemia and medicated hypertension.
As a result of this enquiry, several areas for further research have emerged, including neuropathy and wound healing. Further research is required to determine ideal GTN doses and timeframes for therapy. The major challenges involved are to overcome high variation of individual absorption rates and tolerance. The people most likely to respond well to GTN in this research were nonsmokers, people without diabetes, and those engaging in exercise that was additional to activities of daily living. It was encouraging that some participants with diabetes did respond to GTN and that a third of the highest responders had diabetes. Further research should explore each of these topics, and the effects of temperature, to exploit the value of GTN by enhancing its absorption where possible, and in providing guidance with appropriate patient selection and tailoring of treatment.

Further research to enhance the existing body of knowledge about both normal and pathological TBI values would also be useful. Improvements of toe pressure measurements should involve research about modifiable protocol influences such as cuff size, manual versus automated toe pressure devices, and temperature influences. Researchers should also explore the variability inherent in TBIs and the relationship of TBIs to pathology such as respiratory disease and cardiac output disorders.

This research has also produced practical outcomes. An evidence-based guide to pedal vascular assessment incorporating toe pressures has been developed. Recommendations have been made for vascular assessment of the lower limb to include evidence-based testing, monitoring, and medical referrals for PAD, as well as identification of IADs for the associated CVD risks. In addition, the findings regarding GTN and TBIs have been distilled into clinical recommendations for a summary of how to use GTN for pedal applications, with instructions aimed at addressing the large differences in individuals’ drug absorption and tolerance.

Although they should be regarded as at a preliminary stage, there are recommendations and considerations of this work to assist in applying GTN in clinical practice and also to inform further research. Potentially important benefits to health system economics are implied by the positive findings emerging from the theoretical, empirical, and practical outcomes of this thesis. TBIs are a useful outcome measure for peripheral vascular assessment and for determining the effects of GTN. With further research it will be possible
to improve our understanding of TBIs and the role of GTN in treating pedal ischemia. As a result, individually titrated doses of transdermal GTN, and the use of TBIs to monitor for vascular sufficiency, may become standard components for management of the high-risk foot.
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Appendix A

Ethics Approval Letter

8 November 2011

Ms Sylvia Mc Ara
7 Kennedy Court
WODONGA VIC 3690

Dear Ms Mc Ara,

Thank you for the additional information forwarded in response to a request from the Human Research Ethics Committee (HREC).

The CSU HREC reviews projects in accordance with the National Health and Medical Research Council’s National Statement on Ethical Conduct in Research Involving Humans.

I am pleased to advise that your project entitled “Glycerol Trinitrate In Pediatric Applications” meets the requirements of the National Statement, and ethical approval for this research is granted for a twelve month period from 8 November 2011.

The protocol number issued with respect to this project is 2011/146. Please be sure to quote this number when responding to any request made by the Committee.

Please note the following conditions of approval:

- all Consent Forms and Information Sheets are to be printed on Charles Sturt University letterhead. Students should liaise with their Supervisor to arrange to have these documents printed;
- you must notify the Committee immediately in writing should your research differ in any way from that proposed. Forms are available at www.csu.edu.au/research/forms/hrac_anrep.doc;
- you must notify the Committee immediately if any serious and or unexpected adverse events or outcomes occur associated with your research, that might affect the participants and therefore ethical acceptability of the project. An Adverse Incident Form is available from the website; as above;
- amendments to the research design must be reviewed and approved by the Human Research Ethics Committee before commencement. Forms are available at the website above;
- if an extension of the approval period is required, a request must be submitted to the Human Research Ethics Committee. Forms are available at the website above;

Version 2

FIA

www.csu.edu.au

The Commonwealth Register of Institutions and Courses for Overseas Students (CRICOS) Provider Numbers for Charles Sturt University are 00006F (NSW), 01027G (ACT) and 02509D (ACT).
you are required to complete a Progress Report form, which can be downloaded as above, by 8 November 2012 if your research has not been completed by that date;
• you are required to submit a final report, the form is available from the website above.

You are reminded that an approval letter from the CSU HREC constitutes ethical approval only.

If your research involves the use of radiation, biological materials, chemicals or animals a separate approval is required from the appropriate University Committee.

The Committee wishes you well in your research and please do not hesitate to contact the Executive Officer on telephone (02) 6338 4628 or email ethics@csu.edu.au if you have any enquiries.

Yours sincerely

Julie Hicks
Executive Officer
Human Research Ethics Committee
Direct Telephone: (02) 6338 4628
Email: ethics@csu.edu.au
Cc: Professor Lexin Wang Associate Professor Paul Tinley
Appendix B

International Guidelines Regarding Toe Pressures in Vascular Assessment

Toe pressures are recommended by international authorities in their guidelines for assessment of peripheral vascular supply in high risk populations (Bakker, Apelqvist, & Schaper, 2012; Colaguri, 2013; NHMRC, 2011; Norgren et al., 2007; Rooke et al., 2011).

The table on the following page contains information about these guidelines.
### Table B.1

**International Guidelines Regarding Toe Pressure Measurements**

<table>
<thead>
<tr>
<th>Authority (reference)</th>
<th>Vascular assessment recommendations regarding toe pressures</th>
<th>Links</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wounds international – Best practice guidelines on diabetic foot</td>
<td>When pulses absent or in doubt as to vascular supply, refer to a specialist. Toe pressures are mentioned as a useful adjunct when available.</td>
<td><a href="http://www.woundsinternational.com/pdf/content_10803.pdf">http://www.woundsinternational.com/pdf/content_10803.pdf</a></td>
</tr>
<tr>
<td>TASCII Inter society consensus for management of peripheral arterial disease (Norgren et al., 2007)</td>
<td>TBPI should be used to dx PAD when clinically suspected and when the ABI test is not reliable (Level of Evidence: B).</td>
<td><a href="http://www.ncbi.nlm.nih.gov/pubmed/17223489">http://www.ncbi.nlm.nih.gov/pubmed/17223489</a></td>
</tr>
<tr>
<td></td>
<td></td>
<td><a href="http://circ.ahajournals.org/content/124/18/2020.full.pdf">http://circ.ahajournals.org/content/124/18/2020.full.pdf</a></td>
</tr>
<tr>
<td>International Diabetes Federation – Managing older people with T2D (Colaguri, 2013)</td>
<td>When clinically indicated, it is important to measure toe pressures.</td>
<td><a href="http://www.idf.org/sites/default/files/IDF-Guideline-for-older-people-T2D.pdf">http://www.idf.org/sites/default/files/IDF-Guideline-for-older-people-T2D.pdf</a></td>
</tr>
<tr>
<td>NHMRC guidelines(&quot;International best practice guidelines: Wound management in diabetic foot ulcers,&quot; 2011 p. 17)</td>
<td>“The toe brachial pressure index or toe pressures (using photoplethysmography) are useful adjuncts for assessing foot perfusion if the ABPI is falsely elevated”.</td>
<td></td>
</tr>
<tr>
<td>2011 ACCF/American Heart Association Focused Update of the Guideline for the Management of Patients With peripheral artery disease (Rooke et al., 2011)</td>
<td>TBPI should be used to dx PAD when clinically suspected and when the ABI test is not reliable (Level of Evidence: B).</td>
<td><a href="http://circ.ahajournals.org/content/124/18/2020.full.pdf+html">http://circ.ahajournals.org/content/124/18/2020.full.pdf+html</a></td>
</tr>
</tbody>
</table>
Appendix C

Clinical Protocol for Measurement of TBIs

Toe pressures of the hallux were taken with a portable automated PPG (photoplethysmography) device—the “SysToe” as validated and described by Pérez-Martin et al. (2010).

The following protocol was used to ensure maximum reliability. It is based on the work of Pérez-Martin et al. (2010), Bonham (2011), Bonham, Mueller, and Robison (2010), and Høyer et al. (2013a).

Bonham claims high sensitivity and specificity for TP measurements using this method.

1. Check the toe pressure unit
   - Calibration of TP unit should be ensured by referring to user manual.
   - A pressure of 200 mmHg should be the maximal pressure set for the occlusion cuff inflation.
   - Check TP unit is charged sufficiently for use.

2. Prepare the test room with ambient temperature within range 21–23 °C.

3. Have a flat plinth with 2 pillows available.

4. Lie the patient barefooted, supine on the flat surface and check for comfort. Support head with enough pillows for comfort but do not elevate torso. Heart and feet should be at same horizontal level.

5. Check digital temperature with a reliable thermometer such as the Exergen. If distal toe skin temperature is < 20 °C apply a thermal supplementation such as warmed towels to feet. Consistency in warming protocol is important for future measures so record method and timing. 10–15 min warming recommended when indicated by low skin temperature.
6. Allow a minimum of 10 minutes to rest flat and supine while performing other clinical tasks such as history and medications review, neurological testing, visual inspection, podiatric care and wound care if necessary. Symptoms of arterial insufficiency should be documented.

7. Ensure cuffs and sensor are clean – alcohol wipe.

8. Clean the foot and toes – alcohol wipe.

9. Test for toe cuff size. Loosely apply the base occlusion cuff 1. Choose an occlusion cuff size that is the maximum suitable for both cuffs to fit on the length of the toe allowing a stable attachment of the distal sensor cuff. The standard 25 mm cuff is the default fitting. Use the smaller 15 mm cuff only if the larger one won’t fit. Most women and approximately 25% of men will require the smaller cuff. This occlusion cuff fits around the base of the hallux. Record the cuff size.

10. Apply the sensor cuff- Apply double sided tape to sensor, avoiding any contact of the cuff bladder with the tape as the bladders are easily torn by tape contact. Apply the second sensor cuff with just enough tension to be stable on the hallux with the sensor as low on the on the pulp of the toe as the first cuff will allow. Record which toe was measured first and be consistent with this on repeat measures to assist in measurement standardisation for each side.

11. Take brachial BP in both arms. Identify the highest systolic pressure, and record which arm has the highest pressure. Note - greater than 10mmHg difference in systolic pressure or should trigger medical referral for CV risk management (2 measures of each arm are sufficient).

12. Turn on the toe pressure unit. Insert the highest of the 2 brachial pressures to be stored to perform the index calculation. Start the cuff inflation sequence, and remind the patient at this stage that movement and any talking are to be avoided while the measurements are taken.

13. Perform the measurement three times in immediate succession and record the index each time. See user’s manual for further information about calculating this
within the device. Each measurement can be concluded as soon as the indication of a PPG signal appears.

14. Repeat steps 10 to 13 with the other side hallux.

15. Remove cuffs from test subject and clean – alcohol wipe.

PAD is diagnosed with a reading of < 0.65 on either foot. Medical referral for management of CVD risks should be triggered if PAD is diagnosed. Date and environmental temperature should be documented as variability exists in toe pressures. Lower TP results are associated with colder environments.

Accuracy is improved by serial measures on subsequent occasions. Schedule monitoring when appropriate.
**APPENDIX D**

**Saint Elian Wound Score System (SEWSS)**

<table>
<thead>
<tr>
<th>Anatomical</th>
<th>Aggravating Factors</th>
<th>Affected Tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Location (1-2-3)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Phalanges</td>
<td>Ischemia (0-1-2-3)</td>
<td>Depth (1-2-3)</td>
</tr>
<tr>
<td>2. Metatarsal</td>
<td>0. No (TP&gt;80 mmHg)</td>
<td>1. Superficial (skin only)</td>
</tr>
<tr>
<td>3. Tarsal</td>
<td>1. Mild (TP 60-80)</td>
<td>2. Deep Ulcer (below dermis)</td>
</tr>
<tr>
<td></td>
<td>2. Moderate (TP 40-60)</td>
<td>3. All layers (bone &amp; joint)</td>
</tr>
<tr>
<td></td>
<td>3. Severe (TP&lt;40 mmHg)</td>
<td></td>
</tr>
<tr>
<td><strong>Topographic Aspects (1-2-3)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Dorsal or plantar</td>
<td>Infection (0-1-2-3)</td>
<td>Area (1-2-3)</td>
</tr>
<tr>
<td>2. Lateral or medial</td>
<td>0. No</td>
<td>1. Small &lt;10 cm²</td>
</tr>
<tr>
<td>3. Two or more</td>
<td>1. Mild, erythema &lt;2 cm, induration, tenderness, warmth, purulent discharge</td>
<td>2. Medium 10-40 cm²</td>
</tr>
<tr>
<td></td>
<td>2. Moderate, erythema &gt;2 cm, muscle, tendon, or bone, or joint infection</td>
<td>3. Big &gt;40 cm²</td>
</tr>
<tr>
<td></td>
<td>3. Severe, systemic inflammatory response</td>
<td></td>
</tr>
<tr>
<td><strong>Affected zones (1-2-3)</strong></td>
<td>Odema (0-1-2-3)</td>
<td>Wound Healing Phase (1-2-3)</td>
</tr>
<tr>
<td>1. One</td>
<td>0. No</td>
<td>1. Epithelialisation</td>
</tr>
<tr>
<td>2. Two</td>
<td>1. Periwound</td>
<td>2. Granulation</td>
</tr>
<tr>
<td>3. Entire foot (multiple wounds)</td>
<td>2. Affected leg only</td>
<td>3. Inflammatory</td>
</tr>
<tr>
<td></td>
<td>3. Bilateral secondary to systemic disease</td>
<td></td>
</tr>
<tr>
<td><strong>Neuropathy (0-1-2-3)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0. No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Protective sensation or vibration diminished</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Loss of protective sensation or vibration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Diabetic neuro-osteo arthropathy-Charcot</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>SUBTOTALS</strong></td>
<td><strong>TOTAL</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>/30</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Score Sum</th>
<th>Grade</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10 or 10</td>
<td>Mild</td>
<td>Likely successful wound healing</td>
</tr>
<tr>
<td>11-20</td>
<td>Moderate</td>
<td>Partial foot-threatening; outcome related to “state of the art” therapies used and associated with a good patient biological response</td>
</tr>
<tr>
<td>21-30</td>
<td>Severe</td>
<td>Limb and life threatening; outcome unrelated to “state of the art” therapies because of poor biological patient response</td>
</tr>
</tbody>
</table>

Appendix E

Information and Consent Material for Students in Pilot Study

This two-page appendix contains the information and consent material that was given to the students who participated in the pilot study reported in Chapter 4.

---

Student Information and Consent Form
for GTN Study Participation

Inter- and intra-rater reliability study on assessment measures for the clinical trial—

"Effectiveness of glyceryl trinitrate patches for ischaemia of the feet – a randomized controlled trial"

PhD project of Sylvia McAra
Supervisors- Professor Lexin Wang, Associate Professor Paul Tinley

As final year students in Podiatry at CSU in 2012 and 2013, your participation in a clinical trial is requested.

Your role will include the collection of study data by performing observations, examinations and neurovascular assessments on study subjects being monitored for the effects of glyceryl trinitrate (GTN) on feet in an RCT involving approximately 120 subjects with each subject being assessed a total of 8 times over 6 months throughout 2012-2013.

The aim of the study is to determine whether low doses of GTN have an effect on pedal wounds and neurovascular parameters.

A preliminary reliability trial for the student data collectors of the assessment items spanning 3 hours on a Wednesday afternoon during the final week of your transition clinic will be conducted. 6 potential study subjects will be assessed by each student
on that day using the assessment schedule designed for the larger study. There is an information session beforehand to orient and upskill you for the trial. This can take 2-3 hours, depending on practice time required.

The larger study, the RCT will be conducted within your allocated clinical timeslots. Your participation as final year CSU podiatry students will be acknowledged in all publications resulting from this work.

Your participation in the study in expected to hone your clinical skills in assessment and add to your clinical experience in a unique way. There has been no large scale study on the podiatric effects of GTN reported in the literature to date.

There is the potential that GTN will prove to be a valuable adjunct to wound healing, specifically for pedal wounds in cases of PVD. In the current situation of cardiovascular disease being the chief cause of mortality in Australia and an increasing diabetes incidence worldwide, the implications of positive discoveries in this area are likely to have highly significant implications.

By participating in this study, you will be joining a research team at the forefront of world health science.

Your contribution will be highly valued by myself, Sylvia McAra, as the chief researcher, and also by the research team including Associate Professor Paul Tinley and Professor Lexin Wang.

Please sign this form to indicate your willingness to participate in the project.

Statement of informed consent

I give my consent to be involved as a research participant with the role of student data collector in the above project. I have had an opportunity to ask questions about the study and have understood the requirements. I may ask further questions about the study and my role at any time and can expect to have these addressed as the need arises.

Student’s Name                                          Signature
Date
Appendix F

Information and Consent Material for Patients in Pilot Study

This three-page appendix contains the information and consent material that was given to the patients who participated in the pilot study reported in Chapter 4.

Letterhead for the School of Community Health at Charles Sturt University appeared at the top of this document. It is not reproduced here because of margin constraints.

Trial of Reliability of Students' Measurements of Foot Health

You are invited to consent in participating in the following trial. This trial is a student reliability trial to validate senior year podiatry students for taking measurements in a larger trial;-The larger trial is titled “Effectiveness of glyceryl trinitrate patches for people with impaired circulation in their feet- a randomised controlled study”.

This trial is called “Reliability of podiatry student measurements of foot health”.

The trial period will go for approximately 2 hours on each of 2 days.
Please tick the checkbox relating to each statement when you are clear you understand and are ready consent to each element of the study.

☐ I am to have testing that involves checks of my foot health 6 times on each of 2 visits.

☐ None of the testing is expected to be painful or uncomfortable.

☐ Visits are in November on consecutive Mondays- i.e. 12th at 1.45 and the 19th at 1.00pm.

☐ I am able to withdraw at any time from the study for any reason.

☐ I will in no way be disadvantaged if I choose to withdraw from the study.

☐ My personal information will be kept confidential.

☐ I will be anonymously acknowledged as a study participant in any publications associated with this study.

☐ I have been able to ask questions about the study and my participation and have them answered to my satisfaction

Study participant's name - ________________________________

Signature - _________________________________ Date - ________

Researcher- Sylvia McAra, Signature _________________________

Date - ________
Principal researcher- Sylvia McAra BSc.(Hons) Pod, Clinical Educator

CSU Allied Health Clinic, Corner of Olive and Guinea Streets Albury NSW 2640

Phone -02 60519299   email - smcara@csu.edu.au

Principal Supervisor Dr Lexin Wang ph. 02 69332905 email - lwang@csu.edu.au

Charles Sturt University’s Human Research Ethics Committee has approved this study. For any concerns or complaints about this research, contact:

**Executive Officer - Julie Hicks**  
**Human Research Ethics Committee**  
**Office of Academic Governance**  
**Charles Sturt University**  
**Panorama Avenue**  
**Bathurst NSW 2795**

Phone: (02) 6338 4628  
Fax: (02) 6338 4194
Appendix G

Instructions for Data Collection in Pilot Study

This seven-page appendix contains instructions that were given to the students who participated in the pilot study reported in Chapter 4. This is the amended version, including the VAPS, for the second cohort.

General notes

• Supply a pillow or 2 for the head for patient for their comfort 😊 and lie them down throughout the measurements. It helps to obscure their vision of the neurological measurements and is essential for the accuracy of the vascular measurements.

• The subjects need to be resting recumbent for 10 minutes before vascular measurement, so lie them down first and then perform the other tests during the 10 minutes preparation time. You can put on the brachial and toe cuffs in preparation during this time.

• Visit number and date- initial visit data to determine study eligibility is recorded on a shorter, dark yellow form entitled “Assessment” with just temperature and vascular measurements. For the second and subsequent visits for eligible patch study subjects, the light yellow form called “Patch Data Form” is used with the complete range of study measures adding neurological data to vascular and temperature data.

Instructions and study protocol for assessment items 1-10

A. Neurological tests
1. Pain/10- Ask if the subject has experienced any foot pain in either foot by saying “Have you had any foot pain during the past week?”
Instructions for data collection in pilot study

Showing them the VAPS - visual analog pain scale ruler, ask the patient to indicate what number out of 10 best represents the worst foot pain they have had during the past week in each foot. Suggest that they may hold and align the sliding ruler with either the numbers 1-10 or the faces on the opposite side of the ruler. 😊😊😊 It is necessary to use the VAPS ruler to get reliable results for this question. Distinguish between pain in each foot and score accordingly.

2. **SWMF/10**: Sensation testing with Semmes–Weinstein 10 g monofilament using the Carville protocol, test 10 sites and score here out of 10 for each foot. Clean with Avaguard. Sites are plantar met heads 1, 3, 5, toe apices 1, 3, 5, med. and lat. midfoot, and central heel. In addition, one dorsal site proximal to the cleft between first and second toes dorsally. Avoid callous which invalidates the result 🍎. **Hold the monofilament for 1 second on the spot** while awaiting the client’s response. Check for peaking.

3. **Position sense**: hold hallux by sides, distal to the DIPJ not touching the nail as this invalidates the test. You will also need to hold the lesser toes out of the way. Demonstrate the differing positions of “up” and “down” using the subject’s toe, and then ask them to close their eyes. “Tell me if this is up or down” then move the toe up and down, several times by a small amount of about 4 mm to check if they have position sense. 🧐Record if position sense is P- Present or A- Absent.
Appendix G  Instructions for data collection in pilot study

B. Vascular tests

4. **Brachial systolic pressure.** Take the brachial systolic pressure with one of the desktop Omron automatic BP machines in the clinic, using the subject’s left side. Be aware these desktop ones are easy to drop off the bench as the connecting tube is a little short for our trolley and chair arrangement. Making sure the trolley is right next to the patient chair is a good idea.

5. **Toe pressure and toe pressure indices.** Using a Systoe automatic PPG machine, follow the instructions for “Normal toe” on the laminated sheet within the case.

    Important point - For infection control, take off the old tape and, before placing the new tape on, give the inside of the 2 cuffs a wipe over with Avaguard on a gauze swab. The PPG sensor must be covered with fresh double sided tape and never put straight on the skin. **Do not stick the tape on the inflatable section.** The little cuffs are expensive and can be excessively worn by tearing the adhesive off.

    Cover any wounds with Opsite before testing. If halluces are missing, use the next largest toe.

    Keep the sizing consistent with the size chosen for the initial assessment. (The cuff size affects the measurement significantly, it is raised by about .15 using the smaller cuff. The LARGER cuff is chosen by default if it will fit the patient. Smaller size is needed for most women’s toes.)

    Type in the brachial systolic pressure value to the Systoe machine by pressing “BRA”.

    **Advise your patient they are not to move or talk during the subsequent Systoe testing.**
Take a Toe Pressure by pressing “START” (normal 80-100mmHg) followed by a TPI (toe pressure index) calculation (normal around 0.8-1.0 with a large cuff) from each hallux 3 times in a row. It will retain the brachial input when you press “NEW” for each new reading. Please be sure to record both the systolic pressures (whole numbers) and the TPIs (indexes involving a decimal point) for both feet. Use the white-out tape if you make a mistake with data recording.

The test subject must be still and quiet (not talking) for accurate readings to be obtained. If you get an error message, it is most often due to a problem with the cuff placement. Re-do it as low on the toe as you can and just wrapped around very lightly with complete contact but not tight.

If you get a message that says “Warning – charge too low – use Systoe loader” override this by pressing RET, to completely exhaust the charge before putting the Systoe on the charger. It will do this about 15–20 times. Charging it before it’s completely drained quickly leads to it being unchargeable and it has to go to the repairers in France!

6. **AT pulse**

While the Systoe is working on one foot, do the vascular and temperature tests on the opposite foot. Palpate only the anterior tibial pulse on each foot. Grade 0 for absent, 1 for trace, 2 for diminished, 3 for a normal or strong pulse. Remember on 20% of people it will be located more laterally.
7. **Doppler**

Listen with Doppler for the anterior tibial pulse on each foot. Grade 0 for absent, 1 for monophasic, 2 for biphasic, 3 for triphasic. If anterior tibial pulse is not found, search in the area of dorsalis pedis. If DP is not found, search in the area of posterior tibial pulse. If no pulse is found record 0.

8. **Skin temperatures**

**Apex hallux**

Using the Exergen temperature probes while you are waiting for the Systoe to do the toe pressures, put them on to the scan setting, wipe with Avaguard and gauze just dampened not wet, and take the temperature of each site, beginning with the apex of each hallux by touching lightly on the skin. Be sure to keep your own fingers out of the range of the temperature probe sensor by not holding the toe. This gives falsely elevated readings. Place the probe in line with the sagittal plane of the hallux.

**Skin temperatures- 1st MPJ**

Take the temperature of the medial eminence of the first MPJ from a medial aspect.

**Skin temperatures- MTJ**

The mid foot MTJ measure is taken at 45° to both the transverse and sagittal planes over the area of the naviculo-cuneiform joint.

**Room temperature**

Record the room temperature in your treatment room from the digital thermometer on the desk.
C. Wound assessment

9. **Wound assessment – number of wounds** *(do this while the Systoe is measuring)*

Count the number of wounds on each foot. A wound is any break in the epithelium.

**Wound assessment - grade of wound(s)** Grade the single most severe wound on each foot according to the Saint Elian Wound Score System assessment sheet on the opposite side of the data collection sheet. It results in a score out of 30. Record the locations of the wounds being scored in the patient’s file.

- Wound ischemia is defined by objective signs of vascular insufficiency (non-palpable pulses, monophasic Doppler sounds, toe pressures below 80mmHg systolic or toe pressure indices below 0.65 will be likely to give the most reliable measures — see the toe pressure value table in this folio for ischaemic gradings). However, not infrequently, high toe pressures are found in severe ischaemia. These people are likely to have vessel calcification right down to the toes. They are excluded from the study due to their high pressures.

- Wound infection may be indicated by the presence of one of more of the following:- heat, erythema, swelling, exudate, purulence, and pain.

- Measure each wound with the wound rulers with the camera. Photograph up to 4 wounds on one subject if larger than 2mm in any dimension.
Appendix G  Instructions for data collection in pilot study

Grade the oedema of each foot:

Grade 0.  For nil present
Grade 1.  For periwound oedema only, loss of detail in surface shape on nearby skin
Grade 2.  For the affected leg only- changes to malleoli contours so they are not visible due to oedema
Grade 3.  For significant changes in morphology/shape so that original foot outlines are lost, oedema secondary to systemic disease.

- Grade the phase of healing of the wound by grading the largest percentage of the wound

  Phase 1.  Epithelialisation
  Phase 2.  Granulation-clear, straw coloured exudate is a normal finding in granulating wounds.
  Phase 3.  Inflammatory- distinguish between the latter 2 grades by presence of any slough and quality of exudate- infected or discoloured exudate could be present in the inflammatory phase. Rolled edges are more likely in a chronic inflammatory healing phase. Two types of inflammation, acute and chronic need to be considered here. A wound can be in an inflammatory phase without infection.

10. **Biothesiometer**- You will be assisted if required to perform this measurement.

See the information guide with the VSA- Visual Sensory Analyser machine.

*Thank you for your attention to this information and for your participation, which is highly valued.*
Appendix H

Letters and Information for GPs and Pharmacists

This 20-page appendix contains eight letters that were sent to GPs and pharmacists. All of them relate to the experiment reported in Chapter 5.

Contents

1. Initial contact letter to GPs (4 pages)

2. Letter to GPs regarding intervention group participants (3 pages)

3. Letter to GPs regarding placebo group participants (2 pages)

4. Letter to GPs regarding control group participants (2 pages)

5. Letter to pharmacists regarding placebo group participants (2 pages)

6. GP information letter (2 pages)

7. Final report to GPs regarding their specific patients (2 pages)

8. Progress report for GPs on GTN Study - July 2013 (2 pages)
1. Initial Contact Letter to GPs

Date _____

First contact letter re: “The Effects of Glyceryl Trinitrate on Ischemic Feet”
research at CSU

Dear Dr ______________

I am a podiatrist, working as a Clinical Supervisor at the Charles Sturt University Allied Health Clinic in Albury. I am conducting a study of glyceryl trinitrate (GTN) transdermal patches, applied to feet with arterial insufficiency to assess the effect on neurovascular parameters. This study has received ethics committee approval from the CSU Human Research Ethics Committee.

The purposes of this letter are threefold:

1) To inform you about the study I am conducting,

2) To ask for your ongoing provision of care for patients whom you currently manage, who may be included in the study,

3) To indicate eligibility criteria to enable you to refer appropriate people for inclusion in the study.

First, about the study- As you are aware, glyceryl trinitrate transdermal patches have been available for decades and are a first line drug in the treatment of angina. Despite the proven efficacy of GTN as a local and systemic vasodilator in Coronary Artery Disease and other applications, there are no large scale studies in the literature demonstrating the efficacy of GTN in improving pedal perfusion. Pilot cases treated with GTN at the CSU clinic have however, demonstrated very promising results, including the healing of chronic foot ulceration in diabetics and relief from symptoms of peripheral neuropathy.

There will be 100 patients in this proposed study, divided into either active treatment with GTN (receiving low doses of either 1.25 mg or 2.5 mg), control
or placebo groups. The patches will be cut into appropriate sizes to achieve the required doses and worn continuously, with a new patch applied daily over the 6 months of the study duration. These low doses overcome tolerance issues with GTN. Each patient will be assessed monthly at the clinic over 6 months. The use of GTN is adjunctive in patient care and does not replace or change the podiatric management plan for any patient. Assessment of neurovascular response will be on a range of parameters including symptom profile, clinical examination, neurologic assessment, toe pressure measurements and wound healing.

**Secondly, re your role in the study for your patients:** - You will receive an individualised letter for any patient of yours that is assessed as suitable and willing to participate in the study. You will be informed of this by a letter accompanying the patients in the active treatment group as they present to you for confirmation of their suitability for the study. A courtesy letter also will be sent to inform you if your patient is in the placebo or control group. No prescription or pathology will be required for placebo or control group subjects. Study subjects are informed that they may be given placebo treatment patches. This is a specific item covered in their informed consent as approved by the CSU ethics committee. One in four study subjects will be allocated to the placebo treatment group.

If your patients attend with a letter for inclusion in an active treatment group, please confirm their suitability for treatment with GTN patches and consider the accompanying request for GTN prescription and pathology testing. If for any reason, the patient will not be proceeding as a study participant, I would appreciate communication to this effect.

A prescribing information sheet for practitioners will accompany their individual letters for the active treatment group. If a patient of yours is included for active treatment in the study, their accompanying letter will request you to supply the appropriate pathology request forms and a prescription for Nitro-Dur 5 mg transdermal patches, of 30 X 5 repeats.

All study participants in active treatment groups will require blood tests for routine biochemistry (including renal function and liver enzymes), complete blood picture (CBP) and serum GTN levels. Please direct all pathology test requests to Dorevitch Pathology service at Ramsay place only which is the service collecting. Dorevitch have agreed to bulk bill for services associated with the study. The assay for GTN plasma levels is being done externally by university scientists.
“Nitro-Dur” 5 mg patches are available free of charge for the purposes of the study, avoiding any financial cost to the patient. These patches and the placebo patches will be dispensed only by the Mayo Pharmacy in the Albury Myer Center.

Thirdly, referrals for the study- Patients who demonstrate signs or symptoms of vascular insufficiency, such as diminished or absent foot pulses, foot wounds refractory to healing, foot pain at rest, claudication, a history of chilblains, Raynaud’s or peripheral neuropathy are likely to be suitable for inclusion in the study. I would be pleased to receive referrals from you for such patients for assessment and consideration for inclusion in the study.

If you would like supplementary information including background literature, further information on study inclusion/exclusion criteria or wish to discuss any aspect of the study, please do not hesitate to contact me using the details below.

Sincerely,

Sylvia McAra

Principal researcher- Sylvia McAra BSc.(Hons) Pod, Clinical Educator

phone -02 60519299 0408438610

email - smcara@csu.edu.au

Re: PhD Research Project “Podiatric Applications for Glyceryl Trinitrate”

Principal researcher- Sylvia McAra BSc.(Hons) Pod, Clinical Educator

phone -02 60519299  email - smcara@csu.edu.au

Principal Supervisor Dr Lexin Wang ph. 02 69332905 email - lwang@csu.edu.au
Charles Sturt University’s Human Research Ethics Committee has approved this study. For any complaints or concerns about this research, contact

Executive Officer - Julie Hicks  
Human Research Ethics Committee  
Office of Academic Governance  
Charles Sturt University  
Panorama Avenue  
Bathurst NSW 2795

Phone: (02) 6338 4628  
Fax: (02) 6338 4194
2. Letter to GPs Regarding Intervention Group Participants

Letterhead for the School of Community Health at Charles Sturt University appeared at the top of this document. It is not reproduced here because of margin constraints.

Date
Re:  D.O.B.

Your patient proposed for inclusion in research project

“The Effects of Glyceryl Trinitrate on Ischemic Feet- neurovascular parameters and pain- an RCT”  Active treatment group allocation

Dear Doctor
I wrote to you outlining a study that I am conducting to assess the efficacy of GTN on improving pedal vascular perfusion. At that time I indicated I would write again if a patient of yours is intended for inclusion in the study. Your patient has been assessed, accepted for inclusion and randomised into an active treatment group.

• Would you please confirm this person’s suitability for GTN transdermal patch therapy in regards to their relevant medical history and current medications? I have included the manufacturer’s prescribing information for “Nitro-Dur” and the list of inclusion/exclusion criteria for study subjects for your reference.

• If you can confirm their suitability, the patient will require a prescription for Nitro-Dur 5mg, 30 X 5 repeats. The dose for this patient is a ________ of a 5mg patch ie: ________mg to be applied daily to the dorsum of the ______ foot only. The foot with the lowest vascular perfusion pressure has been selected for the patch application. Both feet will be measured in vascular and neurological parameters over 6 months with 7 measurement episodes.

• This patient will require pathology request forms for Dorevitch Pathology service for the following 4 tests-

1. Kidney function test (EUC)
2. Liver function test (LFT)
3. Full blood examination (FBE)
4. A serum GTN level assay- (to be sent out for independent analysis).

Dorevitch Pathology service has agreed to bulk bill for tests associated with the study and is organised to collect all the samples in the study.
Three important patient information points to review with study subjects

1. Please remind your patient that they are to collect these patches only from the Mayo pharmacy in the Albury Myer Center Mall which is dispensing the “Nitro-Dur” patches free of charge by arrangement for the study participants.

2. Please remind your patient to present with the patches for their next appointment at the University clinic when they will be shown how and where to apply them and baseline measurements taken pre-treatment.

3. Please ensure your patient understands to go to Dorevitch for the blood tests. after commencing using the patches and not before, so that absorption of the patch medication can be measured. The researcher will advise when to go for the test.

- Patients in the study are aware of the study design and agreed to participate on the basis of being blind to receiving active drug or placebo.
- You and your patient will be informed of the results of the study and specific relevance of the outcomes for your patient will be made known to you both.
- Please would you forward the results of the pathology tests on to me when received. Study subjects have agreed to have these results shared in their informed consent to study participation.

Please do not hesitate to contact me if you require any further information.

Thank you very much for your assistance,

Yours sincerely,

Sylvia McAra BSc.(Hons) Pod
Principal researcher - Sylvia McAra BSc.(Hons) Pod, Clinical Educator
phone -02 60519299 email - smcara@csu.edu.au

Re- PhD Research Project “Podiatric Applications for Glyceryl Trinitrate”

Principal researcher- Sylvia McAra BSc.(Hons) Pod, Clinical Educator
Principal Supervisor Dr Lexin Wang ph. 02 69332905 email - lwang@csu.edu.au
Charles Sturt University’s Human Research Ethics Committee has approved this study.

For complaints or concerns about this research, contact:

Executive Officer

Human Research Ethics Committee

Office of Academic Governance

Charles Sturt University

Panorama Avenue

Bathurst NSW 2795

Phone: (02) 6338 4628       Fax: (02) 6338 4194
Date

Dear Doctor __________

Re- your patient ______________________d.o.b. ____________

in the study “The Effects of Glyceryl Trinitrate on Ischemic Feet”

**Placebo group allocation**

This is a courtesy letter to advise you about your patient being included in the abovementioned study. I wrote to you previously, outlining this study, which is assessing the efficacy of GTN on pedal neurovascular parameters. At that time, I indicated I would write again if a patient you are treating meets the eligibility criteria for inclusion in the study.

Your patient is suitable for inclusion in the study and has been randomly allocated to the placebo group.

As a member of the placebo treatment group this person will receive placebo patches which will be dispensed from the Mayo pharmacy, Albury.

All study subjects will be assessed on neurovascular parameters twice in the first month, thence every month for 6 months.

**Patients in the study are aware of the importance of the placebo in the study design and have agreed to participate on the basis of being blind to receiving active drug or placebo as approved by the CSU ethics committee.** This of course, will be subject to your professional discretion, in regards to the patient’s clinical circumstances. If the unlikely circumstances arise in which the patient needs to be informed that they are using a placebo, I would appreciate you communicating this to me.

You and your patient will be informed of the results of the study and specific relevance of the outcomes for your patient will be made known to you both.

Thank you very much for your assistance and cooperation with this project.

Should you require any further information please do not hesitate to contact me.
Yours sincerely,

Sylvia McAra
Sylvia McAra BSc.(Hons) Pod, Clinical Educator
phone -02 60519299, 0408438610
email - smcara@csu.edu.au

Re – PhD Research Project “Podiatric Applications for Glyceryl Trinitrate”
Principal researcher- Sylvia McAra BSc.(Hons) Pod, Clinical Educator
email - smcara@csu.edu.au

Principal Supervisor Dr Lexin Wang ph. 02 69332905 email - lwang@csu.edu.au

Charles Sturt University’s Human Research Ethics Committee has approved this study. For any concerns or complaints about this research, contact:

Executive Officer - Julie Hicks
Human Research Ethics Committee
Office of Academic Governance
Charles Sturt University
Panorama Avenue
Bathurst NSW 2795

Phone: (02) 6338 4628
Fax: (02) 6338 4194
3.  Letter to GPs Regarding Control Group Participants

Date

Dear Doctor,

Re: d.o.b

Your patient has been assessed as suitable for the control group in the study “The Effects of Glyceryl Trinitrate on Ischemic Feet”.

 Significant history-

Assessment results-

This patient qualifies for the study by meeting inclusion criteria including low toe blood pressure index indicative of peripheral arterial disease (<.65). Their lowest result is

Conclusion-

The patient has been requested to attend for measurements of neurological and vascular parameters over 6 months. No treatment is supplied for this patient as a control group study participant. They will not require any pathology testing.

Action requested

No action is requested by you at this stage, however you will be informed as to the results of the study when these are available. This is currently estimated to be at least by end of 2013.

Kind regards,
Sylvia McAra BSc.(Hons) Pod

Principal researcher - Sylvia McAra BSc.(Hons) Pod, Clinical Educator

phone -02 60519299 email - smcara@csu.edu.au

Re: PhD Research Project “Podiatric Applications for Glyceryl Trinitrate”

School of Biomedical Sciences, CSU Wagga Wagga

Principal researcher- Sylvia McAra BSc.(Hons) Pod, Clinical Educator

CSU Allied Health Clinic, Corner of Olive and Guinea Streets Albury NSW 2640

phone -02 60519299 email - smcara@csu.edu.au

Principal Supervisor Dr Lexin Wang ph. 02 69332905 email - lwang@csu.edu.au

Charles Sturt University’s Human Research Ethics Committee has approved this study. For any complaints or concerns about this research, contact

Executive Officer - Julie Hicks
Human Research Ethics Committee
Office of Academic Governance
Charles Sturt University
Panorama Avenue
Bathurst NSW 2795

Phone: (02) 6338 4628
Fax: (02) 6338 4194
5. Letter to Pharmacists Regarding Placebo Group Participants

Dear Pharmacist,

RE:– GTN Patch study subject

The bearer of this letter (name) ___________________________ is a study subject in the PhD research project “Glyceryl trinitrate (GTN) in Podiatric Applications” currently being conducted at Charles Sturt University. They are to use the patch on their ________ foot as directed.

Please dispense them a 6-month supply of the specific study patches, being a box of 192 pieces in the form of 32 patches x 6 sheets pre-cut into quarters.

These patches are held in stock by yourselves for this purpose according to our prior arrangements.

Thank you for dispensing these at no charge to this person.

Thank you very much for your ongoing assistance and cooperation with this project.

Should you require any further information please do not hesitate to contact me.

Yours sincerely,

Sylvia McAra
Sylvia McAra BSc.(Hons) Pod,
Clinical Educator,
Allied Health Clinic
Charles Sturt University
phone -02 60519299 Mobile 0408438610
email - smcara@csu.edu.au
6. GP Information Letter

Date: __________________.

Dear Doctor __________________.

Re: ____________________________ d.o.b __________

Regarding this person’s participation in the current trial at Charles Sturt University “Glyceryl trinitrate in podiatric applications”, I write to you with the following information:

**Significant history**-

**Assessment results**-

**Progress report**-

**Conclusion**-

**Action requested**-
Kind regards,

Sylvia McAra BSc.(Hons) Pod
Principal researcher - Sylvia McAra BSc.(Hons) Pod, Clinical Educator
phone -02 60519299 email - smcara@csu.edu.au
7. Final Report to GPs Regarding Their Specific Patients

Dear Doctor,

Re: d.o.b.

This person has now completed their 6 months of participation in the research trial “Effects of glyceryl trinitrate on ischemic feet” in the group.

Results for this participant:

Effects on toe pressures indicating vascular supply:

Effects on neurological symptoms:

Significance of results

Recommendations

Action requested

Please forward to me the results of this patient’s most recent pathology tests for full blood count, kidney and liver function. This will assist in making conclusions about the effectiveness of this treatment and its wider applicability. Study subjects have given informed consent about sharing their pathology results for the purposes of the study.

Thank you for your support of this patient’s participation in the trial.
Please find an interim report accompanying this letter on the preliminary study findings to date.

As a participating doctor, you will be in receipt of final study results and conclusions as they become available.

Sincerely,

Sylvia McAra

Sylvia McAra BSc.(Hons) Pod

Principal researcher, Clinical Educator

phone -02 60519299 mobile 0408438610 email - smcara@csu.edu.au
8. Progress Report for GPs About the Study – July 2013

Date

Dear Doctor,

In January 2012, data collection commenced for a randomised controlled study on the effects of Glyceryl Trinitrate patches on ischemic feet as a PhD project. Please note our new location, recently changed to the Thurgoona Campus in the new “Wellness Centre” (address above).

The study is now 22 months into the 24 month prospective data collection period. Approximately 90 patients, of the total 120, have been assessed over 6 months using a variety of measures aimed at identifying changes in foot perfusion and symptomatology, including pain experience. Patients have been randomly allocated to one of 4 groups: either active drug treatment with 1.25 mg (quarter of a standard 5mg Nitro Dur patch), 2.5 mg (half a 5mg patch), a placebo group or control group. Active drug or placebo patches have been applied to the dorsum of the foot, changed each 24 hours and worn continuously for 6 months.

Results to date are promising, both with respect to individual clinical observation and positive statistically significant outcome measures with the higher dose group. Patient benefits include improvement both in foot perfusion and reduction in pain experience. While patient numbers associated with the early stage of the trial prevent definitive conclusions, positive clinical and statistical trends are consistent with the literature based theoretical underpinnings on which the study is based. Patient compliance has been high. 5 patients to date have reported a side effect of mild transient headache which did not preclude participation.

A number of ancillary outcome benefits have been suggested by the preliminary data. Although these are encouraging, recommendations necessarily await the analysis at completion of the study. Meanwhile, please be aware that the use of transdermal GTN on lower legs and feet is contraindicated in cases of uncontrolled lower leg oedema, venous incompetence and venous ulceration without adequate compression therapy in place, due to the dilation of veins as well as arteries. The vasodilation effect has the potential to exacerbate any existing venous insufficiency. Compression therapy for
the venous insufficiency may be indicated but with the pressure reduced and individualised for cases with concomitant pedal ischemia.

At the conclusion of the study, a final report and links to publications arisen from the study will be forwarded to you.

Thank you again for your support in regard to your patient’s participation in the study. Your patient referrals and management, particularly in drug prescription, are critical to the success of the study. If there are matters related to the study that require clarification please feel free to contact me or my supervisors.

Sylvia McAra  BSc (Hons) Pod  smcara@csu.edu.au  02 6051 9299 or  mobile 0408 438 610.
Appendix I

Information for Participants in Experiment

This appendix contains information that was provided to participants in the experiment:

Contents

1. Information for all participants.
   This information was provided to all participants, regardless of group of allocation.

2. Information for participants using patches.
   This information was provided to participants in the two intervention groups and the placebo group, but not to those in the control group.

3. Information for participants using the GTN patches.
   This information was provided only to participants in the two intervention groups.

4. Information for participants in the placebo group.
   This information was provided only to participants in the placebo group.
1. Information for All Participants

Note: This information was provided to all participants, regardless of group of allocation.

Letterhead for the School of Community Health at Charles Sturt University appeared at the top of this document. It is not reproduced here because of margin constraints.

Information for Patch Study Participants

1. Introduction

- Glyceryl trinitrate (GTN) is a very safe medication that has been shown to improve circulation. It has been in use for decades as a mainstay of angina treatment because of its effectiveness in improving circulation to the heart.

- Some previous research exists showing the benefits of this medication for improving circulation to feet. There is evidence to suggest that this medication may be useful for many people in reducing the pain and nerve damage associated with diabetes and poor circulation to their feet. Impaired circulation to feet can be associated with problems such as foot ulcers, infection and amputations.

- At Charles Sturt University, there is a study being conducted to clarify the potential of this medication to help people with poor circulation to their feet. The medication will be used as an addition to the high quality, best practice foot care used at the University Clinic. This study will contribute towards knowledge about making these problems easier to prevent or to treat effectively.

For further information please see the following details of the study outline, the participant’s roles and commitments, the involvement of the general practitioners and the benefits of being a participant.
2. Study outline

a. Study Aim – The aim of this study is to show the effect of GTN patch medication on people with reduced circulation in their feet.

b. Study Method – The study will involve about 100 participants who will use a patch on one foot and be monitored for the effects of this over 6 months of use. The effects on the circulation, the nerve function, and on foot pain in both feet will be checked. Some people within the study will be allocated to a placebo treatment group. This makes the study much stronger in its design and the results will be clearer because of this element. The study subjects will not know whether they have the placebo or the active drug, however, the participant’s own General Medical Practitioner will be informed as to whether their patient is on active drug or placebo.

c. Study outcomes - The study is designed to show the effects of the medication on the feet of people with reduced foot circulation. It is expected that the study will enable conclusions to be made regarding the benefits of this treatment.

3. The requirements of study participation

a. Assessment for suitability - In order to be eligible to participate in the study, all potential subjects need to have their suitability checked. This is done through an assessment appointment at the Charles Sturt University podiatry service at the Allied Health Clinic. This is a training clinic for student podiatrists. At an assessment appointment, you will be asked about your medical history, what medications are being taken (please bring a list), and you will receive foot checks. The pulses in the feet will be tested, blood pressure measured in the toes and some tests of nerve functions will be done.

b. Informed consent – To meet ethical requirements and to ensure people understand the study, participants need to sign a consent form. This form goes through each of the elements of the study with opportunities to have any questions answered.

c. Commitments of study participants – 5 possible “to do” items

i. You may be asked to visit your general medical practitioner (GP) at the start of the study with the letter we will give you to check your suitability to be in the study. The GP may then provide you with your prescription for the patches and the pathology request form. The doctor will be requested to bulk bill you for this service so that no costs are incurred. This is however, ultimately up to the Doctor’s discretion. If you have to pay the gap for a standard consultation that the Doctor did not bulk bill, you could be out of pocket for $15.00.
ii. Collect your patches only from Mayo’s Central Albury pharmacy in the Myer centre. These have been donated for the study and are free of charge.

iii. Apply the adhesive patch to the top of one foot each day. You will be advised which foot to use and how to do this when you return for your second visit with the patches.

iv. A single blood test may be prescribed by your GP at the start of the study. The researcher will advise you when to take this test. The test should be done via Dorevitch Pathology where bulk billing has been arranged so as not to incur costs for research participants.

Attend for 7 visits over 6 months while you are using the patches for measurements to be taken. There are 2 measurement visits in the first month, then one visit each month. These visits associated with the measurements for the study are free of charge. It is estimated that these visits might take around 30 minutes.

If you have any wounds on your feet, these may be photographed to assist in monitoring healing.

The visits will be more frequent if you have a wound and some visits will take longer when wound care and measurements also need to be done. The potential side effects of this drug are, in order of their frequency, headaches, flushing, dizziness or light headedness, faintness, nausea or vomiting, or a fast heartbeat. These symptoms are related to the dose of the drug and should rapidly reverse if the patches are removed. If you are suffering these symptoms, remove the patch. Side effects should go away within 2 hours of removing the patch. If symptoms persist or are concerning you, seek medical advice.

If you discontinue using the patches for any reason (including because you simply no longer want to be part of the project), you should inform the researcher as soon as possible. You will not have to give a reason for deciding to leave the project, however the researcher will want to be informed if you have had an adverse reaction to the patches. As well as informing the researcher, please also consult your doctor if necessary.

- Any information collected for this study is treated as confidential. Your name and any identifiable information will not be published. Written and photographic information will be stored securely and later destroyed.
4. If you need podiatry treatment ..........Costs for professional fees

- If podiatry treatment or other assessments are needed in addition to the assessments undertaken for the purposes of the study, the normal Allied Health Clinic charges will apply.
- No costs are to be levied against people for the purposes of the study but if you need extra treatment involving other podiatric services, these services may be supplied within your study appointment if the available time permits, or, if you require this, an additional appointment may be booked for your treatment needs.
- Standard podiatry appointments are charged a fee of $35.00. This is not claimable under Medicare nor through private health funds. A range of other podiatric services including orthoses and surgery are available. Any wound dressings needed will be supplied at cost.
- If you are a resident of NSW and have been referred by a health professional for assessment under the NSW Health Department’s Greater Southern Area Health Scheme (GSAHS), you are eligible for an annual assessment at no charge. People with significant impairment to the circulation or nerve supply of their feet may be eligible for ongoing podiatric consultation and treatment services at no charge under this government scheme with the GSAHS meeting their treatment costs.

5. Benefits of being in the study

- You are likely to benefit from the effects of the medicated treatment on your foot. There may be benefits to both feet.
- Both you and your GP will be informed of the outcomes of the study and the relevance of the findings for your individual case at the conclusion of the study.
- You will be contributing to the growth of understanding in this important area. This is likely to lead to improved prevention and treatment options for people in both the short and long term.

Contacts- CSU Wellness Centre Tharagooona for further information
Phone 02 6051 9299.

and to arrange appointments or 0408438610 to speak directly to the researcher.

Charles Sturt University, Albury, NSW
Re: - Research Project “Effects of Glyceryl Trinitrate on Foot Circulation”

Principal researcher- Sylvia McAra BSc.(Hons) Pod, Clinical Educator email - smcara@csu.edu.au

Principal Supervisor Dr Lexin Wang ph. 02 69332905 email - lwang@csu.edu.au
Charles Sturt University’s Human Research Ethics Committee has approved this study. For any concerns or complaints about this research, contact:

Executive Officer - Julie Hicks
Human Research Ethics Committee
Office of Academic Governance
Charles Sturt University
Panorama Avenue
Bathurst NSW 2795

Phone: (02) 6338 4628
Fax: (02) 6338 4194
2. Information for Participants Using Patches

Note:  This information was provided to participants in the two intervention groups and the placebo group, but not to those in the control group.

— 0 —

Letterhead for the School of Community Health at Charles Sturt University appeared at the top of this document. It is not reproduced here because of margin constraints.

How to use your patches

For study subject (name) ________________________________

This information is to supplement your information document “Information for patch study participants”.

• Place a single patch on the top of the left or right* foot only, within the marked area on the diagram.

• Each patch should stay in place for 24 hours, being worn right through day and night.

• The patches should stay on through normal showering and bathing.

*(Data collector to strike out one whichever of these is not applicable to this study subject)

Patches are for your left foot only*   Patches are for your right foot only.*
• Each day move the new location of the patch slightly in a circular pattern so you use a new area of skin each day. The numbers on the diagram can act as a general guide for each day’s application.

• You can return to the original spot in about 7 days. This is to reduce the chance of a sensitivity reaction to any one area.

• Remove the old patch before applying a new one.

• Wash and dry your hands before and after applying the new patch.

• Replace the patch each day at about the same time.

• Avoid using moisturisers on this area of the foot during the study as this will reduce the adhesion of the patch.

• If the patch doesn’t stick well, place some light, hypoallergenic tape over the area. We can supply this for you if it is needed.
Appendix I  Information for participants in experiment

• If you need a prompt to remind you whether you have changed the patch, write the date on the new one before each new daily application.

• Patches should be stored away from light. Store below 30 degrees. Do not refrigerate.

This information applies to participants in the following project being conducted at Charles Sturt University

“Effects of Glyceryl Trinitrate on foot circulation”

Principal researcher- Sylvia McAra  phone -02 60519299  email - smcara@csu.edu.au
3. Information for Participants Using GTN Patches

Note: This information was provided only to participants in the two intervention groups.

Thank you for participating in our patch study research.

1. Visit your doctor & he/she will consider giving you a script for the patches & a pathology request form. Give us a few days to send the request letter through to your doctor first.

2. Take your script for the patches to Mayo’s Pharmacy Albury Myer City. Centre where it will be Free!

3. When you have collected your patches, contact us to make an appointment for further assessment & instructions on how to use the patches. 0408 438 610 (Sylvia’s mobile phone)

Important! Don’t start the using the patches until you attend at this visit!

4. Have a blood test when instructed by Sylvia at Dorevitch Pathology
Please wait to be told when to take this test!

Any questions please phone Sylvia on 0408438610 or 60519299
4. Information for Participants in Placebo Group

**Note:** This information was provided only to participants in the placebo group.

---

Thank you for participating in our patch study research.

1. Take your letter from us to

   **Mayo’s Pharmacy Albury Myer City Centre**

   where you will be issued with your patches for **Free!**

2. When you have collected your patches, contact Sylvia to make an appointment for further assessment & instructions on how to use the patches.

   📞 0408 438 610  Sylvia

   Any questions please phone Sylvia.
Appendix J

Consent Form for Participants in Experiment

This appendix contains only one item: The consent form for participants in the experiment.

— 0 —

Letterhead for the School of Community Health at Charles Sturt University appeared at the top of this document. It is not reproduced here because of margin constraints.

Participant Consent Form

Title of research project-

A Randomized Controlled Trial on

the Effects of Glyceryl Trinitrate on Ischemic Feet

☐ I have been given the opportunity to ask questions about this study including the use of this medication and the potential side effects, and have the answers explained to me in a way I can understand.

☐ I understand that I am free to withdraw from the study at any time and that if I do wish to withdraw, I can do so without any penalty or disadvantage whatsoever. I may continue to have treatment at the Charles Sturt Clinic even if I discontinue being in the study for any reason.
I understand that any information collected about me is treated as confidential. My name and my information will not be published. Written and photographic information will be stored securely and later destroyed.

I understand that the researcher will contact my GP by letter and may ask him/her to provide me with a prescription for the patches involved in the study and a pathology request for a blood test.

I understand that the prescription is to be filled at the Mayo pharmacy and the blood sample taken at Dorevitch Pathology.

I consent to my pathology results being forwarded to the researcher.

I understand I can contact the researcher or her supervisor at any time for further information about the project.

I understand I can contact the executive officer of the Human research ethics committee with any concerns or complaints about this research.

Study participant’s name - __________________________

Signature - __________________________ Date - ______

If personal assistance is required for the study subject to participate, include carer’s name and co-signature.

Carer’s name - __________________________

Carer’s signature- __________________________ Date- ______

Researcher- Sylvia McAra, Signature ___________ Date-____
Principal researcher- Sylvia McAra BSc.(Hons) Pod, Clinical Educator

Phone -02 6051 9299   email - smcara@csu.edu.au

Principal supervisor Dr Lexin Wang ph. 02 6933 2905 email - lwang@csu.edu.au

Charles Sturt University’s Human Research Ethics Committee has approved this study. For any concerns or complaints about this research, contact:

Executive Officer - Julie Hicks
Human Research Ethics Committee
Office of Academic Governance
Charles Sturt University
Panorama Avenue
Bathurst   NSW  2795

Phone:     (02) 6338 4628
Fax:        (02) 6338 4194
Appendix K

Information About Participants Not Included in Final Analyses

This appendix contains information in two tables about participants who did not complete the study, or whose data were not included in the analyses.

--- 0 ---

Table K.1

*Original Group Numbers and Discontinuers*

<table>
<thead>
<tr>
<th></th>
<th>Low GTN</th>
<th>High GTN</th>
<th>Placebo</th>
<th>Control</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original number</td>
<td>41</td>
<td>31</td>
<td>25</td>
<td>25</td>
<td>122</td>
</tr>
<tr>
<td>Discontinuers</td>
<td>8</td>
<td>7</td>
<td>3</td>
<td>4</td>
<td>22</td>
</tr>
<tr>
<td>% Loss</td>
<td>20%</td>
<td>22%</td>
<td>12%</td>
<td>16%</td>
<td>18%</td>
</tr>
</tbody>
</table>
Table K.2

Information About Participants Who Were Not Included in Final Analyses

<table>
<thead>
<tr>
<th>Group / Duration of participation (months)</th>
<th>Reason given for discontinuation</th>
<th>Additional information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low GTN (n = 8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1</td>
<td>“Transport too difficult.”</td>
<td>Disabling osteoarthritis.</td>
</tr>
<tr>
<td>&lt;1</td>
<td>Scheduling difficulties.</td>
<td>Could not attend monitoring appointments due to absences from the area.</td>
</tr>
<tr>
<td>&lt;1</td>
<td>No reason provided.</td>
<td>Lost to follow up.</td>
</tr>
<tr>
<td>&lt;1</td>
<td>Painful infected ankle ulcer. Did not improve with GTN.</td>
<td>Hospitalised and treated with intravenous antibiotics.</td>
</tr>
<tr>
<td>&lt;1</td>
<td>“Too old + carer duties”.</td>
<td>Advanced age, frailty.</td>
</tr>
<tr>
<td>2½</td>
<td>Mild dementia. Cognition deteriorating.</td>
<td>Transferred to nursing home.</td>
</tr>
<tr>
<td>3½</td>
<td>Preferred not to continue.</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>At Visit 6 reported had not used patches for 4 months. Dropped from analysis.</td>
<td>Experimented with dose increases for neuropathic pain and tried application on both feet – unsuccessful.</td>
</tr>
<tr>
<td>High GTN (n = 7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1</td>
<td>Long travel distance - inconvenient.</td>
<td>—</td>
</tr>
<tr>
<td>&lt;1</td>
<td>Illness.</td>
<td>Dizziness and light-headedness, which persisted after GTN cessation.</td>
</tr>
<tr>
<td>2½</td>
<td>Broken leg.</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>Acute knee injury.</td>
<td>—</td>
</tr>
<tr>
<td>1</td>
<td>Medical need to commence prophylactic GTN for angina.</td>
<td>Cardiac issues.</td>
</tr>
<tr>
<td>1¼</td>
<td>Referred for vascular investigation.</td>
<td>—</td>
</tr>
<tr>
<td>1¼</td>
<td>Ineligible with unstable BP.</td>
<td>—</td>
</tr>
<tr>
<td>Placebo (n = 3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1</td>
<td>Preferred not to continue. “Too old.”</td>
<td>—</td>
</tr>
<tr>
<td>&lt;1</td>
<td>Had advice from vascular surgeon preferring other intervention.</td>
<td>—</td>
</tr>
<tr>
<td>3½</td>
<td>Had cardiac and leg stenting surgery.</td>
<td>Was on prior wait list for this surgery.</td>
</tr>
<tr>
<td>Control (n = 4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1</td>
<td>No reason given.</td>
<td>History of PTSD and alcohol abuse.</td>
</tr>
<tr>
<td>&lt;1</td>
<td>No reason provided.</td>
<td>Lost to follow up.</td>
</tr>
<tr>
<td>5</td>
<td>Medical reasons - unwell.</td>
<td>Kidney transplant.</td>
</tr>
</tbody>
</table>
Figure 6.1

Vascular Assessment Flowchart

Developed by Sylvia McAra, CSU, November 2014

The flowchart on the following page is referred to in Section 6.3.2 of Chapter 6. It is provided as a guide to evidence-based vascular assessment that incorporates vascular tests with acceptable sensitivity and specificity in order to enable appropriate diagnosis, referral, and stratification of risk status for peripheral artery disease (PAD) and cardiovascular disease (CVD). Monitoring recommendations are determined from the risk status.

Abbreviations used overleaf

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABI</td>
<td>Ankle brachial index</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>CVT</td>
<td>Capillary venous filling time</td>
</tr>
<tr>
<td>IAD</td>
<td>Interarm difference</td>
</tr>
<tr>
<td>PAD</td>
<td>Peripheral artery disease</td>
</tr>
<tr>
<td>TBI</td>
<td>Toe brachial index</td>
</tr>
</tbody>
</table>
**Step 1. History**
Screen for signs and symptoms of PAD and the conditions italicised in the adjacent box.
Note: 50% of PAD is asymptomatic.

**Step 2. Visual assessment**
Know the visual signs, but appreciate that all are low in sensitivity and specificity for PAD except for Buerger’s sign.

**Step 3. Palpation** - Pulses should be taken but have low inter-rater reliability, and low sensitivity and specificity, especially in the presence of the conditions in italics in the box at Step 1 above. CVT and temperature are also low in sensitivity and specificity.

**Step 4. Doppler sounds and wave forms**
Monophasic Doppler is high (but not perfect) in sensitivity and specificity, including in the presence of the conditions listed in italics in the box at Step 1 above.
Biphasic sounds do not indicate lack of pathology.
Interpret triphasic results with caution in the presence of other risk factors and signs because false “normals” occur.

**Step 5. ABI/TBI**
Progress to one of these tests if any abnormality found previously.

**Step 6. Action** – Dependent on assessment results.

---

**Diabetes, neuropathy, advanced age, kidney disease**
Presence of the above conditions causes pulses and the ABI to be very low in sensitivity.
Respiratory and cardiovascular diseases, especially cardiac arrhythmia are associated with high fluctuations and overall reduction and in peripheral perfusion.

Buerger’s sign is 100% sensitive for severe PAD.

Absent or reduced pulses are a preliminary indication of PAD but have low specificity and sensitivity, especially in the presence of related disease.

Perform Doppler sound and waveform analysis (best sensitivity and specificity for detecting PAD).

Monophasic - Abnormal
Biphasic - Ambiguous
Triphasic - Normal
If triphasic with no other signs, no further vascular assessment required at this time.

The TBI test is recommended in preference to ABI if any of the conditions in italics at Step 1 are present, or if ABI > 1.2.
TBI <.71 is abnormal with large cuff or <.77 with small cuff.
Screen for IADs and refer when abnormal >10 mm Hg.

Abnormal results - Refer immediately due to high associated CVD risks.
Borderline results - Inform GP; reschedule for serial repeat measurements and then routine reassessment.
Normal results - Schedule routine reassessment - vascular review at least annually for people with diabetes and other risk factors. Annual vascular review for others aged over 50.
Appendix M

Recommendations Regarding Use of Pedal Glyceryl Trinitrate

This material is referred to in Section 6.4.7 of Chapter 6. It is intended to be given to medical practitioners if prescription of GTN in pedal applications is requested by a podiatrist or other health practitioner.

Contents

Introduction
Indications
Instructions
Precautions and contraindications
Contact
References

Introduction

Transdermal GTN at a dose of 2.5 mg appears effective for increasing pedal vascular pressure as emerged from a recent Level III 2 experiment.

A moderate effect on improvements in vascular perfusion was found in the recent experiment conducted at Charles Sturt University on pedal use of GTN. The study had 100 participants with low toe pressures, 57 of whom received GTN patches. The study used continuous treatment, with doses of 1.25 and 2.5 mg. Outcomes were evaluated with toe pressures as the primary outcome measure. Significant improvement for the 2.5 mg dose had occurred at 1 month and at 65 months, and for 1.25 mg at 5 months. Publication of findings is expected in 2015.

Note contraindications and precautions before prescribing.
Recommendations for Use of Pedal GTN

One of the biggest challenges with the use of GTN is the 10-fold difference in absorption rates, determined by studies on the skin of the chest for cardiac applications. The absorption from the periphery in people with PAD is unknown. This necessitates case-by-case titration of the dose from a starting point up until the desired effect is achieved. Too large a dose results in tolerance within 24 hours and the increased likelihood of side effects, the commonest of which is headache. A mild transient initial headache is not, however, necessarily prohibitive as it indicates that absorption of a potentially effective dose has occurred. Headaches occurred in 25% of participants in the study, were reported as mild to very mild, typically lasted for two days and were not a barrier to ongoing participation for any participant. Local rather than systemic effects appear to predominate using these small doses in the periphery. The following recommendations need to be validated with further testing, but are the starting point for further investigation, having been compiled from the existing evidence.

Indications

Reduced vascular perfusion, foot wounds associated with ischemia, painful neuropathy.

Instructions

These instructions are aimed at balancing the dose of GTN for each individual between tolerance and side effects. Information for clients and a chart to assist in monitoring effects to guide titration are available in separate documents.

- GTN is available as Nitro-Dur 5 in circular 5 mg patches for transdermal delivery, and this is the lowest dose available. (Nitro-Bid is unsuitable because its presentation is in a sachet that cannot be cut to the required appropriate small doses.)
- A 2.5 mg patch daily for 24 hours on the dorsum of one foot is the ideal initial dose. This keeps the dose under the threshold for development of drug tolerance when vascular perfusion is unimpaired at the site of application.
- Because absorption is a function of both pedal perfusion and cardiac output, effective doses vary, as do the doses at which tolerance occurs.
- If relief or improvement in toe pressure is not achieved by the initial dose, increase the dose by 1.25 mg every 4–7 days until the desired effect of improved perfusion, wound...
healing, or relief of neuropathic pain is achieved, or until a mild headache is noted. Cutting the circular patch into quarters allows for titration in 1.25 mg increments.

- No rest period is required for doses of 2.5 mg or less as tolerance has been shown not to occur at these low doses (Wei et al., 2011).
- Doses over 2.5 mg have been associated with tolerance when used for periods greater than 24 hours. However, this has not been tested in people with PAD. Using doses higher than 2.5 mg should involve a rest period of 8–14 hours out of 24 unless impaired absorption increases the tolerance threshold.
- Standard doses of simple analgesics may be indicated if necessary to manage headache. Headaches typically resolve within 2 days.
- After determining a single dose that is effective and comfortable for the individual, this dose may be split to use on both feet (Rayman, Baker, & Krishnan, 2003).
- Monitoring of toe pressures is helpful to guide dosage for optimal therapy. (This is available through a high-risk foot service such as the Charles Sturt University podiatry service, Thurgoona. Phone 02 6051 9299 for appointments.)
- The sites of application should be rotated to a new area of skin on the dorsum of the foot over a 7-day cycle. See diagram in client use instructions.
- Skin reactions occur in about 5% of users. Strict site rotation can assist in management of mild rash. This therapy should be discontinued if a rash persists.
- For painful neuropathy, pulsed therapy of up to 3 months trial for effectiveness is suggested. (Literature suggests the number needed to treat is 4–5 for an effect with painful neuropathy.) The ideal timeframes for pulsed therapy are not yet known.
- Treatment effect is probably ameliorated with exposure to cold. Keeping feet warmly insulated is important, noting that people with sensory neuropathy and/or aged > 80 years are likely to have reduced temperature sense in their feet and thus be not fully aware of the thermal status of their feet.
- In cold temperatures, the use of high quality, loose topped socks seems helpful in improving the effectiveness of GTN treatment. Footwear should be of a quality sufficient to insulate against cold and large enough to accommodate the extra bulk of the socks without constriction of the feet. Two pairs of thinner socks may be superior to one thick pair and may provide better thermal management and insulation.
Physical activity in addition to activities of daily living was the strongest predictor of response to GTN. Therefore, it would probably be useful to prescribe exercise along with GTN patches for PAD.

**Precautions and contraindications**

See product information for the complete list of practitioner information regarding the use of glyceryl trinitrate, which includes unstable blood pressure and PDE4 inhibitors as contraindications: http://www.merck.com/product/usa/pi_circulars/n/nitro-dur/nitrodur_pi.pdf

Safety and efficacy of transdermal GTN for in cases of venous ulceration is not established and may be harmful for these types of wounds as there is a preferential dilation of venous vessels over arterial vessels that could increase venous incompetence and may exacerbate this type of wound. People with venous ulceration and venous insufficiency were excluded from the CSU study for this reason.

A precaution regarding an as-yet unreported possibility of this treatment having an association with increased incidence of Charcot’s neuroarthropathy of the foot, should be considered in GTN therapy. If people with diabetic neuropathy display a significant increase in temperature, i.e., “hot spots” greater than 4 °C, they should be considered at risk and monitored regularly for potential Charcot’s neuroarthropathy or ulceration. Regular temperature monitoring is the standard recommendation for monitoring for potential ulceration and neuroarthropathy, and should be adhered to with particular vigilance if GTN therapy is used on the foot in cases of diabetic neuropathy.

No people in the GTN treated groups of the study developed varicose symptoms, hot spots, or Charcot’s neuroarthropathy. Temperature monitoring at three sites was conducted at each monthly visit. In a separate study, one person with long-term (18 month) use of GTN patches developed superficial telangiectasia.

**A balance between risks and benefits should be considered on an individual basis.**

**Further study about the effects of the use of GTN in pedal applications is expected to add to the specificity of these recommendations.** See accompanying patient information with a record-keeping table for monitoring and titration guidance.

Refer to prescribing information and publications of the author for further information.
Appendix M  Recommendations for Use of Pedal GTN  337

Contact

Sylvia McAra BSc (Hons) Podiatry
smcara@csu.edu.au
ph  02 6051 9299
PhD project:  Toe Pressures and GTN in Pedal Ischemia
Bldg 715, Ellis Street
Community Engagement and Wellness Centre
Charles Sturt University
Thurgoona  NSW  2640.

References


Appendix N

GTN for Feet: Client Information

This material is referred to within Section 6.4.7 of Chapter 6. It is intended for medical practitioners to give to patients when prescribing GTN in pedal applications. It may have been provided to the medical practitioners by a referring podiatrist or other health practitioner if a prescription for GTN was requested.

— 0 —

How to Use GTN Patches for Feet

GTN patch medication may improve foot circulation and reduce foot pain.

Name ________________________________ Date ______________

• The start dose is 2.5 mg for the left or right foot*.

  2.5 mg is half of the circle of the whole 5 mg Nitro-Dur patch.

• The patch needs to be removed from its packet, then cut in half with sharp scissors.

• To act as a prompt to remind you whether you have changed the patch, write today’s date on the new one before each daily application.

• Place the leftover half back in the packet and fold it over twice. This can be used on the following day.

• Place the patch on the flat top surface of the left or right foot*.

* Strike out one whichever of these is not applicable to this person as this information is given.
• Each patch should stay in place for 24 hours, being worn right through day and night.

• The patches should stay on through normal showering and bathing.

• Each day move the location of the patch in a circular pattern so you use a new adjacent area of skin each day. This is to reduce the likelihood of a rash occurring. The numbers on the diagram can act as a general guide for each day’s application.

![Diagram showing patch application areas on feet]

• Apply the new patch before removing the old one to help to place it adjacent to the old site.

• You can return to the original spot after about 7 days.

• Replace the patch each day at about the same time.

• Wash your hands after handling the patch. It is important to remove any traces of the sticky side from hands to minimise side effects such as headache.

• A mild headache is a common temporary effect of this medication in the first 2 days.

• The effect of the patch disappears 2 hours after removal. If you have any unexpected effects from the use of the patch, remove the patch, discontinue use, and see your doctor.
• Avoid using moisturisers on this area of the foot as doing so will reduce the adhesion of the patch.

• If the patch doesn’t stick well, place some light, hypoallergenic tape over the area. Micropore or Mefix are examples of suitable tape.

• Patches should be stored away from light. Store below 30 °C, but do not refrigerate.

• Discontinue if a rash or skin irritation develops.

• This information is a supplement to product information that comes in the manufacturer’s packet. Refer to that information for more details.

Your prescribing doctor will need your feedback to find the correct dose for you. Fill in the accompanying record chart and take it with you when being reviewed by your doctor for this treatment. After your individual baseline dose is determined on one foot, both feet may be treated.

For further advice contact your prescribing doctor or:

Podiatry Department
Community Engagement and Wellness Centre
Charles Sturt University
Building 715 Ellis Street
Thurgoona NSW 2640

Phone 02 6051 9299
Appendix O

GTN Pedal Use: Client Monitoring Table

This material is referred to within Section 6.4.7 of Chapter 6. It is intended for medical practitioners to give to patients when prescribing GTN in pedal applications. It may have been provided to the medical practitioners by a referring podiatrist or other health practitioner if a prescription for GTN was requested.

When patients are given this material for recording information about their use of GTN, they will be provided with instructions for completing the charts. This will include how pain is to be assessed, which will be by means of scoring out of 10 using a visual analogue pain scale (VAPS).
## GTN Pedal Use: Client Monitoring Table

<table>
<thead>
<tr>
<th>Time</th>
<th>Action</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>00:00</td>
<td>Start</td>
<td>Begin monitoring session</td>
</tr>
<tr>
<td>00:15</td>
<td>Adjust</td>
<td>Adjust settings as needed</td>
</tr>
<tr>
<td>01:00</td>
<td>Stop</td>
<td>End monitoring session</td>
</tr>
</tbody>
</table>

*Note: Detailed monitoring data not included in this summary.*
GTN* patches - Table to record effects

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>GTN Dose Left</th>
<th>GTN Dose Right</th>
<th>Total dose</th>
<th>Pain Score Left</th>
<th>Pain Score Right</th>
<th>Comments</th>
<th>Pain relief medication dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Take this chart to your doctor when you are returning for review of your treatment with GTN patches. Your doctor may recommend a different dose after your trial period. The correct dose is important for effectiveness and is individually determined with trialing.

*GTN - Glyceryl trinitrate