Exercise prescription for improving inflammatory and glucose regulatory outcomes in inactive Indigenous Australian and Caucasian men

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I

Amy Eileen Mendham

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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 nor material which 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 acknowledgment 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 the thesis be accessible for the purpose of study and research in accordance with the normal conditions established by the University Librarian for the care, loan and reproduction of the thesis.*

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Signature                                      Date

* Subject to confidentiality provisions as approved by the University
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Publications resulting from this Thesis

The following publications are in support of this thesis:


# Abbreviations

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<th>Description</th>
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<tr>
<td>ABS</td>
<td>Australian Bureau of Statistics</td>
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<tr>
<td>ACSM</td>
<td>American College of Sports Medicine</td>
</tr>
<tr>
<td>Akt/PKB</td>
<td>Protein Kinase B</td>
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<tr>
<td>AMPK</td>
<td>5’ Adenosine Monophosphate-Activated Protein Kinase</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
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<tr>
<td>BMI</td>
<td>Body Mass Index</td>
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<tr>
<td>BSA</td>
<td>Bovine Serum Albumin</td>
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<tr>
<td>CHO</td>
<td>Carbohydrate</td>
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<tr>
<td>CI</td>
<td>Confidence Interval</td>
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<tr>
<td>CK</td>
<td>Creatine Kinase</td>
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<tr>
<td>COX</td>
<td>Mitochondrial Complex</td>
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<tr>
<td>CRP</td>
<td>C - Reactive Protein</td>
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<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
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<tr>
<td>CYC</td>
<td>Cycle Ergometry</td>
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<tr>
<td>DXA</td>
<td>Dual-Energy X-Ray Absorptiometry</td>
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<tr>
<td>EDTA</td>
<td>Edetate Calcium Disodium Agent</td>
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<tr>
<td>FFA</td>
<td>Free Fatty Acids</td>
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<tr>
<td>FO</td>
<td>Fluoride Oxalate</td>
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<tr>
<td>GLUT4</td>
<td>Glucose Transporter 4</td>
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<tr>
<td>GPS</td>
<td>Global Positioning System</td>
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<tr>
<td>GXT</td>
<td>Graded Exercise Test</td>
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<tr>
<td>β-HAD</td>
<td>3-Hydroxyacyl CoA Dehydrogenase</td>
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<tr>
<td>HbA1c</td>
<td>Glycosylated Haemoglobin</td>
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<tr>
<td>HDL</td>
<td>High Density Lipoprotein</td>
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<tr>
<td>HIIT</td>
<td>High Intensity Intermittent Training</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>Homeostasis Model-Insulin Resistance</td>
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<tr>
<td>HPA</td>
<td>Hypothalamic-Pituitary-Adrenal</td>
</tr>
<tr>
<td>HR&lt;sub&gt;max&lt;/sub&gt;</td>
<td>Maximum Heart Rate</td>
</tr>
<tr>
<td>hs</td>
<td>High Sensitivity</td>
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<tr>
<td>IA-FM</td>
<td>Intra-Abdominal Fat-Mass</td>
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<tr>
<td>IKK</td>
<td>Ikappa B Kinase Beta</td>
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<tr>
<td>IFG</td>
<td>Impaired Fasting Glucose</td>
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<tr>
<td>IGT</td>
<td>Impaired Glucose Tolerance</td>
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<tr>
<td>IL</td>
<td>Interleukin</td>
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<tr>
<td>JNK</td>
<td>Jun N-terminal kinase</td>
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<td>LDL</td>
<td>Low Density Lipoprotein</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>MI</td>
<td>Myocardial Infarction</td>
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<tr>
<td>mRNA</td>
<td>Messenger Ribonucleic Acid</td>
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<tr>
<td>NF-κB</td>
<td>Transcriptional Factor Nuclear Pathway</td>
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<tr>
<td>NRF</td>
<td>Nuclear Respiratory Factor</td>
</tr>
<tr>
<td>OGGT</td>
<td>Oral Glucose Tolerance Test</td>
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<tr>
<td>OXPHOS</td>
<td>Oxidative Phosphorylation</td>
</tr>
<tr>
<td>PDH</td>
<td>Pyruvate Dehydrogenase E1α</td>
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<tr>
<td>PGC-1α</td>
<td>Peroxisome Proliferator-Activated Receptor Gamma Coactivator-1α</td>
</tr>
<tr>
<td>p53</td>
<td>Protein 53</td>
</tr>
<tr>
<td>Ra</td>
<td>Receptor agonist</td>
</tr>
<tr>
<td>RM</td>
<td>Repetition Maximum</td>
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<tr>
<td>RPE</td>
<td>Rating of Perceived Exertion</td>
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<tr>
<td>RPM</td>
<td>Revolutions per minute</td>
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<tr>
<td>RT</td>
<td>Room Temperature</td>
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<tr>
<td>SSG</td>
<td>Small-Sided Games</td>
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<td>SST</td>
<td>Serum Separated Tube</td>
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<tr>
<td>SIRT1</td>
<td>Sirtuin 1</td>
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<tr>
<td>T2DM</td>
<td>Type 2 Diabetes Mellitus</td>
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<tr>
<td>TB-FFM</td>
<td>Total-Body Fat-Free Mass</td>
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<td>TB-FM</td>
<td>Total-Body Fat-Mass</td>
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<td>Tfam</td>
<td>Mitochondrial DNA Transcription Factor A</td>
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<td>TNF-α</td>
<td>Tumor Necrosis Factor - Alpha</td>
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<td>VO2</td>
<td>Oxygen Uptake</td>
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<td>VO2max</td>
<td>Maximal Oxygen Uptake</td>
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<td>VO2peak</td>
<td>Peak Oxygen Uptake</td>
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<tr>
<td>WBC</td>
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<tr>
<td>WC</td>
<td>Waist Circumference</td>
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<tr>
<td>WHR</td>
<td>Waist to Hip Ratio</td>
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<tr>
<td>Wmax</td>
<td>Maximal Aerobic Workload</td>
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## Symbols & Subunits

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<thead>
<tr>
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<tr>
<td>±</td>
<td>Plus or minus</td>
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<tr>
<td>°C</td>
<td>Degrees Celsius</td>
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<tr>
<td>%</td>
<td>Percent</td>
</tr>
<tr>
<td>cm</td>
<td>Centimetre</td>
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<tr>
<td>d wk(^{-1})</td>
<td>Days per week</td>
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<tr>
<td>h</td>
<td>Hour</td>
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<tr>
<td>Hz</td>
<td>Hertz</td>
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<tr>
<td>Kg</td>
<td>Kilogram</td>
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<tr>
<td>Kg m(^2)</td>
<td>Kilograms per meter squared</td>
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<tr>
<td>m</td>
<td>Metre</td>
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<td>min</td>
<td>Minute</td>
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<tr>
<td>mL</td>
<td>Millilitre</td>
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<tr>
<td>mL·Kg(^{-1})·min(^{-1})</td>
<td>Millimetres per kilogram per minute</td>
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<tr>
<td>min wk(^{-1})</td>
<td>Minutes per week</td>
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<tr>
<td>mmol</td>
<td>Millimole</td>
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<td>mM</td>
<td>Millimolar</td>
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<td>ms</td>
<td>Millisecond</td>
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<td>Millivolt</td>
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<td>µL</td>
<td>Micro Litre</td>
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<td>µM</td>
<td>Micromolar</td>
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Abstract

This thesis compared the acute and chronic effects of rugby-specific small-sided games (SSG) and cycle ergometry (CYC) on inflammation and glucose regulation in middle-aged, inactive Indigenous Australian and Caucasian men. The initial investigation compared the inflammatory and glucose responses between Indigenous Australian and Caucasian men following an acute bout of CYC. Despite being matched for fitness and body composition the Indigenous men had elevated resting tumor necrosis factor (TNF)-α and glucose values compared to the Caucasian men. These differences may have contributed to the suppressed post-exercise anti-inflammatory (interleukin (IL)-6, IL-1 receptor agonist (ra) and cortisol) response observed in Indigenous men following exercise (p<0.05). Moreover, there was a similar post-exercise (0-240 min) decrease in glucose between groups (p<0.05), which highlights the value of acute moderate-intensity exercise to be completed daily to assist with long-term improvements in glucose, irrespective of ancestry.

The second study compared the acute inflammatory and glucose regulatory response within and between SSG and CYC in Caucasian men. Results demonstrated that both SSG and CYC were sufficient to stimulate an acute anti-inflammatory response, through the post-exercise elevation of IL-6, IL-1ra and cortisol (p<0.05). Neither SSG nor CYC stimulated a post-exercise pro-inflammatory TNF-α, IL-1β and C-reactive protein (CRP) response. The novel findings were that the acute bout of SSG elevated post-exercise IL-6 and increased blood glucose disposal when compared with an intensity- and duration-matched CYC condition.

The third study assessed changes in pro- and anti-inflammatory cytokines, aerobic capacity and body composition following 8-weeks of either SSG or CYC training, compared to an inactive control (CON) condition within Caucasian men. Cycle ergometry and SSG training
were effective in reducing CRP and total body fat-mass (TB-FM), and increasing aerobic capacity (p<0.05). However, the SSG were more effective in reducing resting IL-6 and leptin concentrations and increasing total-body fat-free mass (TB-FFM) (p<0.05).

The fourth study assessed systemic and skeletal muscle glucose regulatory and mitochondrial adaptations following 8-weeks of either SSG or CYC training, compared to an inactive CON condition. Both SSG and CYC showed similar improvements in reducing glucose area under the curve (AUC), total body fat-mass and increasing aerobic capacity (p<0.05). However, SSG training was more effective in increasing TB-FFM, strength and decreasing insulin AUC (p<0.05). Furthermore, there were no changes (p>0.05) in the content of skeletal muscle proteins associated with glucose regulation (i.e. glucose transporter 4 and protein kinase B) and mitochondrial biogenesis (i.e. Peroxisome proliferator-activated receptor gamma coactivator-1α, Sirtuin 1, p53, mitochondrial complex subunits I-V and transcription factors). These results suggest that short term (8 weeks) exercise training in previously inactive Caucasian men does not increase the content of these respective proteins. Rather, it is suggested that an increased efficiency of these proteins and enzyme activity may be responsible for the systemic glucose regulatory adaptations in both CYC and SSG conditions.

The fifth study investigated the acute effects of CYC and SSG on inflammation and glucose regulation within Indigenous Australian men. There was no increase in cortisol in either condition (p>0.05), and both conditions stimulated a post-exercise anti-inflammatory response demonstrated through an immediate post-exercise increase in IL-6, followed by a peak in IL-1ra at 60 min post-exercise (p<0.05). Neither CYC nor SSG stimulated a post-exercise pro-inflammatory (TNF-α, IL-1β and CRP) response (p>0.05). Considering the similar physiological response the higher perceived enjoyment of SSG compared to CYC
(p<0.05) suggest that this mode of exercise may be a more palatable and socially-inclusive exercise intervention for Indigenous Australian men.

Finally, the sixth study assessed the impact of a 12-week sports-based (inclusive of rugby SSG) intervention on glucose regulation systemic inflammation and anthropometry, compared to an inactive CON within inactive Indigenous Australian men. The sport-based training stimulated improvements in aerobic capacity, body mass, body mass index, waist circumference, waist to hip ratio, insulin AUC, estimated insulin sensitivity, insulin resistance and plasma leptin concentration (p<0.05). These results highlight the potential for implementing sports-based training methods for improving clinical risk-factors associated with type 2 diabetes mellitus (T2DM) in normo-glycemic, but insulin resistant Indigenous Australian men.

Collectively, findings from this thesis demonstrate that based on the acute physiological (study 1) and cultural differences between Indigenous Australian and Caucasian men, there is a benefit for implementing culturally appropriate training interventions designed for the specific needs of the community. Specifically, study 5 established a greater perceived enjoyment when completing an acute bout of rugby small-sided games than compared to continuous cycling within Indigenous Australian men. The present thesis demonstrated that rugby SSG elicited similar (study 5) or greater (study 2) responses as dose-matched CYC, promoting glucose disposal and a systemic anti-inflammatory milieu within the post-exercise period in both inactive, middle-aged Indigenous Australian (study 5) and Caucasian men (study 2). Furthermore, study 3 demonstrated that rugby-specific SSG’s is a more effective than CYC training in reducing pro-inflammatory markers IL-6 and leptin and increasing TB-FFM in inactive Caucasian men. Additionally, study 4 revealed that rugby-SSG’s training
was also an effective alternative to CYC for decreasing metabolic risk-factors associated with the prevention of T2DM and cardiovascular disease (CVD), in inactive Caucasian men. There were however, no changes in the content of skeletal muscle proteins associated with glucose regulation and mitochondrial biogenesis. Importantly, incorporating SSG’s across a variety of sports and group training activities into an exercise training program for Indigenous men (study 6) improved metabolic, anthropometric and aerobic capacity variables.
CHAPTER ONE

Introduction
Chapter 1: Introduction

1.1: Background

Physical inactivity and obesity are common risk factors for chronic disease (World Health Organization, 2013). The increasing prevalence of cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM) within both Indigenous and non-Indigenous Australians has been reported alongside increasing levels of obesity and physical inactivity (Australian Bureau of Statistics, 2007-2008b, 2011-2013). These data suggest exercise interventions that improve body composition may assist in reducing the burden of chronic disease (Bartels, Davidson, & Gong, 2007; Hu, Tuomilehto, Silventoinen, Barengo, & Jousilahti, 2004; Panagiotakos, Pitsavos, Chrysohoou, Kavouras, & Stefanadis, 2005). Despite the poorer lifestyle and higher prevalence of T2DM and CVD amongst Indigenous Australian populations (Australian Institute of Health and Welfare, 2011), there is little published research reporting on the efficacy of specific exercise interventions that may ameliorate potential disease risks. However, regardless of ancestry, a physically inactive lifestyle can lead to alterations in body composition, including increased adiposity and decreased muscle mass (Alberti, Zimmet, & Shaw, 2006; Lakka & Laaksonen, 2007). Fat-to-muscle-mass ratio has shown to be associated with altering systemic inflammatory and glucose regulatory mechanisms within the blood and skeletal muscle; all of which are interrelated mechanisms known to contribute to the development of metabolic and cardiovascular abnormalities i.e. T2DM and CVD (Alberti et al., 2006; Arany, 2008; De Luca & Olefsky, 2008; Fantuzzi, 2005; Lanza & Sreekumaran Nair, 2010; Magliano et al., 2008; Pradhan, Manson, Rifai, Buring, & Ridker, 2001; Prior et al., 2009).

Chronic inflammation is an important risk factor for several clinical diseases, including CVD and T2DM. Findings from cross-sectional investigations have shown an inverse relationship between physical activity or aerobic capacity and levels of chronic systemic inflammation in
men, women, and children across both healthy and diseased participants (Aronson et al., 2004a; Aronson et al., 2004b; Church et al., 2002; Mohamed-Ali et al., 1997; Panagiotakos et al., 2005; Pischon, Hankinson, Hotamisligil, Rifai, & Rimm, 2003; Roytblat et al., 2000; Visser, Bouter, Mcquillan, Wener, & Harris, 1999). Furthermore, numerous studies demonstrate that exercise decreases pro-inflammatory cytokines and the risk associated with onset of chronic disease development (Aronson et al., 2004a; Panagiotakos et al., 2005).

Accordingly, there is increasing interest in the application of specific exercise interventions to reduce the chronic inflammatory state in an attempt to ameliorate disease risk (Beavers, Brinkley, & Nicklas, 2010; Donges, Duffield, & Drinkwater, 2010; Lavie, Church, Milani, & Earnest, 2011; Nicklas, You, & Pahor, 2005). A primary prevention strategy of CVD and T2DM involves increasing engagement in physical activity to promote changes in body composition and accordingly restore the balance between pro- and anti-inflammatory mediators (Arend, 2002; Hotamisligil, 2006; Ouchi, Parker, Lugus, & Walsh, 2011). Finally, an improved inflammatory state has direct influences on glycaemic control and insulin signalling, which are also risk-factors of developing metabolic and cardiovascular disorders (Arend, 2002; Hotamisligil, 2006; Ouchi et al., 2011).

Skeletal muscle is the major site for glucose disposal in lean healthy glucose tolerant individuals (Defronzo et al., 1981b; Holloszy & Coyle, 1984; Kristiansen, Gade, Wojtaszewski, Kiens, & Richter, 2000). Following a meal, approximately one third of ingested glucose is absorbed by the liver and the remaining by peripheral tissues, primarily by skeletal muscle via insulin dependent mechanisms (Cherrington, 1999; Zierath, Krook, & Wallberg-Henriksson, 2000). The postprandial hyperglycemia stimulates insulin secretion from the pancreas and the rise in plasma insulin concentrations stimulates glucose uptake and subsequent metabolism in skeletal muscle (Cherrington, 1999; Zierath et al., 2000). In a state
of insulin resistance (i.e. T2DM and obese individuals) insulin stimulated glucose disposal in skeletal muscle is markedly impaired (Defronzo, Ferrannini, & Simonson, 1989; Zierath et al., 2000). The decreased insulin-stimulated glucose uptake is due to impaired insulin signalling and post-receptor intracellular defects, including impaired glucose transport and phosphorylation, and reduced glucose oxidation and glycogen synthesis (Defronzo et al., 1989; Eckardt, Taube, & Eckel, 2011; Pradhan, 2007; Zierath et al., 2000). The exact mechanisms that lead to the development of insulin resistance in skeletal muscle are not fully understood. However, it is proposed that a combination of factors associated with systemic inflammation and defects in mitochondrial functioning collectively contribute to reduced insulin release, insulin sensitivity and signalling within the muscle are involved (Egan & Zierath, 2013; Finck & Kelly, 2006; Kim, Wei, & Sowers, 2008; Lira, Benton, Yan, & Bonen, 2010). Such developments lead to increased systemic concentrations of glucose and insulin in a fasting and fed state, precipitating the onset of insulin resistance and associated development of T2DM (Abdul-Ghani & Defronzo, 2010; Kitabchi et al., 2013).

Given the role of mitochondria in regulating cellular glucose uptake and providing energy balance, positive mitochondrial adaptations to exercise may provide a method to improve glucose tolerance (Hoppeler & Fluck, 2003). As such, the preservation of aerobic capacity and skeletal muscle mass through exercise training can ameliorate metabolic abnormalities. In part, the aforementioned improvements in glucose regulation may relate to exercise-induced molecular remodelling of the skeletal muscle mitochondria (Egan & Zierath, 2013; Hoppeler & Fluck, 2003). Indeed, the benefits of exercise for chronic disease prevention have traditionally been associated with systemic adaptations and improvements in clinical risk factors (i.e. fat-mass, muscle mass, inflammatory state, aerobic capacity, lipid profile) associated with glucose regulation and/or insulin sensitivity (Alberti et al., 2006; Durstine,
Gordon, Wang, & Luo, 2012; Laaksonen et al., 2002). In part, these clinical benefits have been attributed to metabolic and mitochondrial remodelling within skeletal muscle (Egan & Zierath, 2013).

To date, the majority of research has focused on these respective clinical health benefits in relation to continuous, aerobic-based exercise training, such as cycle ergometry (Egan & Zierath, 2013; Garber et al., 2011). Such exercise stimuli predominately involves lower-body, concentric muscular contractions capable of reducing fat-mass, and improving the chronic inflammatory state, cardiovascular function, glucose regulation and mitochondrial biogenesis (Bijker, De Groot, & Hollander, 2002; Goodyear & Kahn, 1998; Hawley & Lessard, 2008; Irrcher, Adhihetty, Joseph, Ljubicic, & Hood, 2003; Libardi, De Souza, Cavaglieri, Madruga, & Chacon-Makahil, 2012). Accordingly, the American College of Sports Medicine (ACSM) recommend that ‘apparently healthy’ adults engage in moderate-intensity cardiorespiratory exercise for $\geq 30$ min$^{-1}$ on $\geq 5$ d$^{-1}$ wk$^{-1}$ for a total of $\geq 150$ min$^{-1}$ wk$^{-1}$, vigorous-intensity cardiorespiratory exercise training $\geq 20$ min$^{-1}$ on $\geq 3$ d$^{-1}$ wk$^{-1}$ for a total of $\geq 75$ min$^{-1}$ wk$^{-1}$, or a combination of moderate- and vigorous-intensity exercise (Garber et al., 2011). In particular, a recent ACSM position stand recommends incorporating interval training (varying intensity of fixed intervals over a single exercise bout) as an additional approach for exercise prescription (Garber et al., 2011). Of note, this particular review of exercise prescription acknowledges the effectiveness of traditional prolonged cycle ergometry based modes, in addition to recommending that interval-based training may provide similar or additional benefits to physical health. For example, interval training can result in similar or greater adaptations in aerobic capacity, glucose regulation, mitochondrial activity and pro-inflammatory states, when compared to continuous exercise in healthy adults (Croft et al., 2009; Gormley et al., 2008; Helgerud et al., 2007; Nybo et al., 2010; Whyte, Gill, & Catheart,
2010). However, it also acknowledges the clear lack of evidence within inactive cohorts (as opposed to young, healthy participants), and that further research is required to fully understand the health benefits across different modes of intermittent specific exercise.

Aligned with above, a common barrier for Indigenous Australians to participate in regular exercise is the lack of facilities in rural and regional communities to promote the conventional approach to exercise training through gym-specific methods of aerobic (i.e. cycle ergometry or running) and resistance-based exercises. These types of structured exercise training programs provide physiological benefits specific to inflammation, glucose regulation and body composition (Donges et al., 2013; You, Arsenis, Disanzo, & Lamonte, 2013; You & Nicklas, 2008). However, the limitation with implementing these conventional programs within regional Indigenous Australian populations is that it does not represent the communities’ physical activity patterns, interest and/or understanding of community involvement into one’s health (Thompson & Gifford, 2000; Thompson, Gifford, & Thorpe, 2000). Furthermore, Indigenous communities need to be provided with an opportunity for encouraging community engagement and ownership over an exercise program. As such, the efficacy of traditional gym-based exercise as sustainable and effective mode to reduce disease risk in Indigenous populations may be limited. In particular, team sports such as Australian football and rugby league football codes are of highest popularity amongst Indigenous Australians (Neesham & Garnham, 2012). Whilst the efficacy of sport specific small sided games (SSG) have been investigated in healthy and clinical northern European cohorts (Krstrup et al., 2010a), this approach for improving inflammation and glucose regulation in regional Indigenous and Caucasian Australians has not been investigated. Thus investigating exercise prescription through SSG may provide an opportunity of evidence-based research for exercise prescription, specific to the cultural interests within these regional communities.
Sport-specific SSGs incorporate frequent bouts of high-intensity efforts, separated by low-intensity movements over prolonged durations (Bijker et al., 2002; Campbell et al., 2014) and may be prescribed to provide the benefits associated with both intermittent and continuous training (Coffey & Hawley, 2007; Krstrup et al., 2010a). Accordingly, soccer-specific SSG training has been of recent research interest in inactive Caucasian men and women, which has shown positive functional (i.e. cardiorespiratory and strength), body composition (i.e. increased muscle mass and decrease fat-mass) and skeletal muscle (i.e. increased capillary density, fibre type and metabolic efficiency) adaptations (Krstrup et al., 2010a; Krstrup et al., 2010b; Krstrup et al., 2009). However, no data is available on rugby-specific SSG and its effect on systemic inflammatory adaptations and/or systemic and skeletal muscle glucose regulatory adaptations within inactive, middle-aged men.

The potential health benefits resulting from both cycle ergometry and SSG training across inactive populations include the acute responses in glucose utilisation and the release of anti-inflammatory cytokines. It is assumed the regularity of this acute stress via exercise training promotes improved chronic adaptation relating to glucose regulation, systemic inflammation, body composition and mitochondrial biogenesis (Gleeson, 2013; Lanza & Sreekumaran Nair, 2010). In turn, such physiological adaptations are suggested to improve clinical risk factors for the overall prevention of chronic disease development (Ishii, Yamakita, Sato, Tanaka, & Fujii, 1998; Jurca et al., 2005; Unwin, Shaw, Zimmet, & Alberti, 2002). However, whether rugby specific SSG can provide additional improvements for the aforementioned parameters relating to physical health remain to be fully elucidated. Notably, the acute and chronic demands of soccer specific SSG for untrained individuals has been well established within recent years (Andersen et al., 2014; Krstrup et al., 2009; Krstrup, Dvorak, Junge,
Bangsbo, 2010c; Randers et al., 2010b). However, no studies have described the respective acute demands of rugby specific SSG compared to traditional laboratory based modes such as cycle ergometry. A greater understanding of these exercise-induced responses would assist in explaining the chronic adaptations as related to systemic inflammation, glucose regulation and mitochondrial biogenesis within Caucasian men. Moreover, despite high rates of chronic disease, obesity and physical inactivity no acute or chronic exercise programs have been assessed in Indigenous Australian men, with a focus on systemic inflammation and glucose regulation.

1.2: Statement of problem

The literature on the prevention and protection from chronic disease development (i.e. CVD and T2DM) through exercise-based interventions has been shown to be promising. Previous studies have described the effects of continuous and/or intermittent training (i.e. cycle ergometry and running), interventions upon glucose control, inflammation and mitochondrial biogenesis in blood and skeletal muscle within Caucasian populations. However, no studies have described the effects of exercise interventions upon the inflammatory and glucose regulatory adaptations that can defend against chronic disease development within Indigenous Australian populations. Rugby specific SSG may be a practical and culturally appropriate intervention aimed at improving health and fitness within inactive populations. Since sport-specific SSGs may be a potent exercise intervention for improving aspects of health (blood pressure, cholesterol, body composition) in different ancestry groups, further research investigating the specific underlying blood and muscle-based mechanisms including mitochondrial biogenesis, glucose control and inflammation is required. Therefore the purpose of the present thesis was to examine the acute and chronic inflammatory and glucose
regulatory effects of exercise, particularly rugby specific small-sided games in inactive, middle-aged Indigenous Australian and Caucasian men.

1.3: Research aims & hypotheses

The present thesis investigated the:

1) Comparison of the glucose and inflammatory responses to an acute cycle ergometry (CYC) bout between Indigenous and Caucasian Australian men;

2) Acute effects of SSG and CYC on systemic glucose regulatory and inflammatory responses within Caucasian men;

3) Inflammatory and body composition adaptations associated with 8 weeks of SSG and CYC training within Caucasian men;

4) Effects of 8 weeks of CYC and SSG training on systemic glucose regulation and content of proteins association with glucose regulation and mitochondrial biogenesis in Caucasian men;

5) Acute effects of SSG and CYC on systemic glucose and inflammatory responses within Indigenous Australian men; and

6) Inflammatory, glucose regulatory and body composition adaptations associated with 12 weeks of sports-specific training within Indigenous Australian men.
Study 1

*Differences in post-exercise inflammatory and glucose responses between inactive Indigenous Australian and Caucasian men completing a single bout of cycle ergometry.*

**Aim:** This study compared the acute inflammatory and glucose responses following CYC in sedentary Indigenous Australian and Caucasian men, matched for aerobic capacity and body composition.

**Hypothesis:** It was hypothesised that the reported disparity in health status between these two cohorts may be reflected in different resting metabolic and inflammatory variables. These resting metabolic and inflammatory disparities may further reflect differences between ancestry groups in the post-exercise inflammatory and glucose response to CYC.

Study 2

*Differences in the acute inflammatory and glucose responses between rugby-specific small-sided games and cycle ergometry in middle-aged, inactive Caucasian men.*

**Aim:** This study compared the acute inflammatory and glucose responses within and between rugby-specific SSG and CYC in inactive, middle-aged Caucasian men.

**Hypothesis:** It was hypothesised that when matched for intensity, SSG would not differ in the post-exercise inflammatory response to CYC. Further, the acute inflammatory response in both modes would be indicative of an acute increase in anti-inflammatory markers following exercise.
Study 3

*Rugby-specific small-sided games training is an effective alternative to improve the chronic inflammatory state compared to continuous cycle ergometry in middle-aged, inactive Caucasian men.*

**Aim:** This study assessed changes in pro- and anti-inflammatory cytokines, aerobic capacity and body composition following 8-weeks of either rugby-specific SSG or CYC training in middle-aged, inactive Caucasian men.

**Hypothesis:** It was hypothesised that when matched for training load and intensity both SSG and CYC training will equally improve the anti- and pro-inflammatory state, body composition and aerobic capacity.

Study 4

*Rugby specific small-sided games training is an effective alternative to continuous cycle ergometry for improving glucose regulation in middle-aged, inactive Caucasian men.*

**Aim:** This study assessed systemic and skeletal muscle glucose regulatory responses following 8-weeks of either rugby-specific SSG compared to CYC training in middle-aged, inactive Caucasian men.

**Hypothesis:** It was hypothesised that SSG would be an effective alternative to CYC training for inactive, middle-age Caucasian men, capable of eliciting positive changes in glucose regulatory risk-factors and skeletal muscle proteins associated with the prevention of T2DM and CVD.
Study 5

Rugby-specific small-sided games is as effective as cycle ergometry at stimulating an acute inflammatory and glucose response in middle-aged, inactive Indigenous Australian men.

Aim: This study investigated the acute effects of CYC and rugby-specific SSG on inflammation and glucose responses within an Indigenous Australian population.

Hypothesis: It was hypothesised that when matched for intensity, rugby-specific SSG would not differ in the post-exercise inflammatory response to CYC. Further, the acute inflammatory response in both modes would be indicative of an acute increase in anti-inflammatory markers following exercise.

Study 6

A 12-week sports-based exercise program for inactive Indigenous Australian men improved clinical risk factors associated with type 2 diabetes.

Aim: This study assessed the impact of a 12-week sports-based exercise intervention on glucose regulation, as well as anthropometric and inflammatory markers associated with the prevalence of T2DM in Indigenous Australian men.

Hypothesis: It is hypothesised that sports-specific exercise training sessions, particularly inclusive of SSG’s and boxing, may be an effective approach for increasing physical activity and improving glucose regulatory and inflammatory risk factors associated with the development of T2DM.
1.4: Limitations

It is acknowledged that there are several limitations. These include:

- Due to the subject population being inactive middle-aged males and free from known diseases, caution should be encouraged if transferring the findings of this study to other subject cohorts i.e. females, children, adolescents, and the elderly;

- Participants were required to complete food and physical activity diaries. This required participants to follow a controlled diet 24 h pre and post exercise testing sessions and to also refrain from exercising externally to the study. All efforts were made to ensure compliance with the study objective; however, the accuracy and honesty of the provided information may not have been completely controlled;

- Participants were required to refrain from exercise and strenuous physical activity between exercise protocols, and particularly in the 48 h prior to biopsy collection; however, this cannot be completely enforced;

- Participant compliance/adherence with the designed testing sessions cannot be completely controlled; however, all efforts were made to ensure maximum compliance and adherence with all exercise testing sessions;

- Participants inflammatory responses may at times reflect stimuli other than the exercise protocol i.e. influenza or infection; however, all attempts were made to ensure participants are free from such illnesses during testing and data collection;

- The cultural sensitivity required for conducting research within Indigenous Australian populations meant some participants may be precluded based on individual cultural requirements;
1.5: Delimitations

- All baseline testing and exercise protocols were conducted in a closed and controlled environment within the respective Institutional Exercise Laboratory;

- Participants were familiarised for all studies, ensuring subject familiarity with testing procedures, measures, and equipment;

- Participants completed the exercise testing procedures (study 1, 2 and 5), 2 h oral glucose tolerance test (OGTT), venous blood collection (study 3, 4 and 6) and resting muscle biopsies (study 4) at the same time of day to minimise any potential diurnal variation and effects on muscle and venous blood samples; all measures were conducted by the same tester utilising identical equipment;

- Participants underwent identical preparation for the resting OGTT in studies 2 and 3; accordingly, participants replicated the first exercise session in standardising the exercise stimulus prior to the administration of the post-exercise OGTT;

- Participants were provided with a dietary journal and were required to record their diet in the 24 h prior to the collection of resting biopsy. Participants replicated this diet in the 24 h prior to each of the respective exercise protocols;

- Participants were required to abstain from caffeine 3 h and alcohol 24 h prior to baseline and exercise testing sessions;

- Participants were required to fast for 10-12 h prior to pathology collection during baseline testing;

- A respected Indigenous community member was present during all testing procedures involving Indigenous Australians.
CHAPTER TWO

Review of Literature
2.1: Overview

This review of literature initially discusses the prevalence and outcomes of physical inactivity and obesity within Indigenous and non-Indigenous Australians. Further discussion then leads into the repercussions of chronic disease associated with physical inactivity and obesity, and the associated mechanisms of systemic inflammation, glucose regulation and mitochondrial dysregulation. The focus is then directed toward exercise as an approach to prevent the onset of chronic disease associated with the acute and chronic adaptations of continuous and intermittent-based exercise in relation to pro- and anti-inflammatory markers, glucose regulation and associated mitochondrial biogenesis. Additionally, attention is directed to specific literature involving exercise and Indigenous populations, with emphasis on Indigenous Australians. Finally, SSG as a method for exercise prescription in Indigenous Australian and Caucasian men is further discussed in association with the potential for inducing favourable acute and chronic inflammation and glucose regulatory adaptations. Figure 2.1 provides a conceptual overview of these topics to be presented in a subsequent section of this review of literature.
Figure 2.1 Conceptual overview of the review of literature
2.2: Outcomes of physical inactivity and obesity

2.2.1: Prevalence of physical inactivity and obesity in Indigenous and non-Indigenous Australians

The prevalence of overweight (Body Mass Index (BMI) \( \geq 25 \text{ kg m}^2 \)) and obese (BMI \( \geq 30 \text{ kg m}^2 \)) Australians aged 18 y and over has shown a continual increase, from 56.3% in 1995, to 61.2% in 2007-2008 and 62.8% in 2011-2012 (Australian Bureau of Statistics, 2011-2013). These rates vary according to geographical location, with men who reside in inner regional, outer regional and remote areas (74.4%) of Australia being more likely to be overweight or obese compared with men living in major cities (67.7%). Although not to the same extent, this pattern is also consistent within women (Australian Bureau of Statistics, 2011-2013).

Physical inactivity is reported as a key modifiable risk factor for obesity, with evidence reporting reductions in waist circumference (WC), BMI and waist to hip ratio (WHR) having significant health benefits observed via a greater prevention of chronic disease development (Canuto, Mcdermott, Cargo, & Esterman, 2011; Janssen, Katzmarzyk, & Ross, 2004; Vazquez, Duval, Jacobs Jr, & Silventoinen, 2007). In Australia there has been an increase in sedentary behaviour amongst adults since 2001. After adjusting for age, 36% of adults (18 y and over) were sedentary in 2007-2008, an increase of 4% since 2001. Within this timeframe (2001-2008) the proportion of people who exercised at moderate levels decreased by 2%, while the proportion who exercised at low or high levels remained stable (Australian Bureau of Statistics, 2007-2008b). These increasing rates of sedentary behaviour and obesity have been associated with the increasing prevalence of non-communicable diseases such as, CVD (i.e. heart disease and stroke), T2DM and the development of some cancers (i.e. breast and colon) (Australian Bureau of Statistics, 2007-2008b; World Health Organization, 2013).
Accordingly, physical inactivity and obesity are individually and collectively defined as common risk factors for chronic disease development (World Health Organization, 2013). Within the last two decades there has been an increasing prevalence of diagnosed cases of the chronic diseases such as T2DM and CVD within the Australian population, which reflects global trends (Alberti et al., 2006; World Health Organization, 2013; Zimmet, Alberti, & Shaw, 2001). Predictions are that this increased prevalence of disease rates will continue to rise, particularly in developed countries, including Australia (Shaw, Sicree, & Zimmet, 2010). For example, it has been suggested that the world prevalence of diabetes among adults (20-79 y) in 2010 (6.4%) would increase to 7.7% by 2030, with developed countries increasing by 20% compared to 69% in developing countries (Shaw et al., 2010). Similarly, it is suggested a high proportion of the Australian population is living with undiagnosed T2DM. As evidence, a study by Dunstan et al. (2002) investigated 2 h OGTT on 11,247 subjects from 42 different living regions in Australia to determine the prevalence of T2DM, impaired glucose tolerance (IGT), and impaired fasting glucose (IFG). Results indicated that the prevalence of T2DM was 8.0% in men and 6.8% in women, and an additional 17.4% of men and 15.4% of women had IGT or IFG (Dunstan et al., 2002). In comparison, the Australian Bureau of Statistics reported the prevalence of T2DM at 4% in 2007-08, which largely underestimates the prevalence of T2DM within the Australian community and suggests the potential for a significant increase in prevalence rates within the upcoming years (Dunstan et al., 2002).

Diabetes is a disease known to predispose individuals to the future development of atherosclerosis, leading to the development of CVD and the occurrence of myocardial infarction (MI) (Hansson & Libby, 2006; Ridker, Buring, Cook, & Rifai, 2003). In 2004-05, CVD was the leading cause of mortality in Australia accounting for more than 36% of all
Chapter 2: Review of Literature

deaths. Cardiovascular disease is the most expensive health condition in Australia, involving a direct health care expenditure of $5.5 billion, in 2000-01, which accounted for 10.9% of allocated recurrent health system expenditure (Australian Bureau of Statistics, 2004-2005). In 2007-08, indirect health care expenditure involved an additional 18% of the Australian population reporting other long term conditions of the circulatory system including, angina, stroke, and hypertensive disease (Australian Bureau of Statistics, 2007-2008a). These results indicate epidemiological data from the Australian population in its entirety. However, when these statistics are separated into Indigenous and non-Indigenous Australian populations, a skewed prevalence exists for higher disease rates of chronic disease at a younger age within Indigenous compared to non-Indigenous Australian populations (Vos, Barker, Begg, Stanley, & Lopez, 2009).

Physical inactivity and obesity are preventable risk-factors for the development of chronic diseases, including T2DM and CVD, which are all highly prevalent in Indigenous Australian populations (Trewin & Madden, 2005). Comparatively, in 2004-05 Indigenous Australians were 1.5 times likely as non-Indigenous Australians to report being sedentary and obese (Trewin & Madden, 2005). Additionally, obesity/overweight and physical inactivity contributed to 11% and 8.4% of the Indigenous disease burden, respectively (Australian Institute of Health and Welfare, 2011). These data supports the trend for elevated prevalence of chronic disease development in Indigenous populations at a younger age than Caucasian Australians; with prevalence rates among the Indigenous populations aged 35-44 y being almost as high as non-Indigenous Australians aged 55 y or over (Thomson, Midford, Debuyst, & Macrae, 2011; Trewin & Madden, 2005). Moreover, the worldwide incident rates of T2DM have been on the rise in the last 30 y and though based on limited data, seem to disproportionately affect Indigenous groups in both developed and non-developed countries.
Chapter 2: Review of Literature


Taken collectively, the increasing prevalence of CVD and T2DM within the Indigenous and non-Indigenous Australian populations are reported alongside increasing levels of obesity and physical inactivity. These data suggest that initiatives such as exercise should be encouraged to assist with increasing levels of physical activity, improving measures of body composition and overall assisting with decreasing the future burden of chronic disease (Bartels et al., 2007; Hu et al., 2004; Panagiotakos et al., 2005). However, despite the poorer lifestyle and higher prevalence of T2DM and CVD amongst Indigenous populations (Australian Institute of Health and Welfare, 2011), there is little published research reporting physical activity related methods to ameliorate potential disease risks.
2.2: Role of systemic inflammation in the development of chronic disease

2.2.1: Overview

Damage to any tissue triggers a cascade of events that leads to the repair of a wound (Martin & Leibovich, 2005). This cascade involves the infiltration of inflammatory cytokines, neutrophils, macrophages and mast cells, as a means to dispose of and eliminate necrotic and damaged cells (Martin & Leibovich, 2005). Clearly, this inflammatory response is crucial in fighting infection and forms a major element to innate and adaptive immunity (Martin & Leibovich, 2005). When acute illness, injury or sepsis is absent, yet elevation of blood-based inflammatory cytokines is marked, a state of chronic inflammation is present and known to participate in mechanisms relating to chronic disease development (Pedersen, 2011a). In particular, the imbalance of pro- and anti-inflammatory cytokines creates a state of chronic systemic inflammation by increasing the plasma concentrations of pro-inflammatory cytokines and suppressing the concentration of anti-inflammatory cytokines (Hansson & Libby, 2006; Petersen & Pedersen, 2006). Furthermore, circulating mediators of inflammation, many of which are secreted by adipocytes and adipose tissue-derived macrophages, participate in the mechanisms of vascular insult and impaired insulin signalling (Dandona, Aljada, & Bandyopadhyay, 2004; Hotamisligil, 2003). Accordingly, mounting evidence highlights the role of adipose tissues in the development of a systemic inflammatory state and the overall contribution to obesity-related cardiovascular and metabolic risk (Dandona et al., 2004; Hotamisligil, 2003).

Systemic inflammation precipitates a group of lifestyle-related chronic diseases such as CVD and T2DM (Pradhan, 2007; Ridker, Rifai, Stampfer, & Hennekens, 2000b; Shoelson, Lee, & Goldfine, 2006). Obesity and a high fat diet activates the Jun N-terminal kinase (JNK) and
Ikappa B kinase beta/nuclear factor kappa B (IKK/ NF-κB) pathways in adipocytes, hepatocytes and associated macrophages (Pradhan, 2007). This inflammatory pathway further initiates an elevation of pro-inflammatory cytokines (i.e. tumor necrosis factor (TNF)-α, interleukin (IL)-1β IL-6), which stimulate the hepatic synthesis of acute phase protein C-reactive protein (CRP) and signals an overall chronic inflammatory state (Gabay & Kushner, 1999; Shoelson et al., 2006; Tilg & Moschen, 2006). Furthermore, a dose dependent increase in CRP mRNA and protein expression has been observed with increasing concentrations of leptin (0-400 ng mL$^{-1}$), which is also known to be produced in adipocytes (Singh, Hoffmann, Wolk, Shamsuzzaman, & Somers, 2007).

In response to this chronic inflammatory state, the anti-inflammatory marker IL-1ra is elevated in an attempt to suppress IL-1β and retain homeostasis of the innate immune system (Chernoff et al., 1995; Petersen & Pedersen, 2005). Furthermore, anti-inflammatory markers such as adiponectin and IL-10 are known to improve endothelial function, insulin sensitivity and down regulate the synthesis of pro-inflammatory cytokines (Arita et al., 1999; Bouassida et al., 2010). However, these anti-inflammatory markers are suppressed in obese and insulin resistant individuals, and in coordination with increased pro-inflammatory markers, result in a sub-clinical inflammatory state and the development of metabolic and cardiovascular abnormalities (Arita et al., 1999; Bouassida et al., 2010). As outlined in Figure 2.2, inflammatory pathways in macrophages or adipocytes can be initiated by extracellular mediators such as cytokines, lipids, glucose, or by intracellular stresses such as endoplasmic reticulum stress or excess reactive oxygen species production by the mitochondria (Wellen & Hotamisligil, 2005). Thus, a balance must be obtained between metabolism and inflammation both systemically and within the cell. Prior to discussing the role of exercise on inflammatory markers and glucose regulation, some discussion of the role of respective inflammatory and
glucose regulatory makers associated with obesity, physical inactivity and subsequent disease progression is required.

**Figure 2.2:** Model of overlapping metabolic and inflammatory intracellular and extracellular mediators in adipocytes or macrophages.

2.2.2: TNF-α

TNF-α is produced both in adipose tissue and skeletal muscle, and may function in an autocrine manner to inhibit insulin signalling and glucose transport (Plomgaard et al., 2005). As evidence, TNF-α expression is increased in adipose tissue of obese and type 2 diabetic animal and human models (Plomgaard et al., 2005). Conversely, when TNF-α activity is low, either via biochemical or genetic manipulation, an improved state of insulin sensitivity is observed (Hotamisligil, 2003; Plomgaard et al., 2005). Notably, this is not a consistent finding, with previous research reporting that whilst TNF-α tended to be higher, no significant difference in concentrations were evident between healthy controls (3.1 ±1.8 pg.mL⁻¹) and those with T2DM (4.9 ±2.7 pg.mL⁻¹) (Netea et al., 1997). Regardless of these inconsistencies, elevated concentrations of TNF-α negatively regulate the insulin signal transduction for whole body glucose uptake, leading to a strong association with the development of a pro-atherosclerotic state and aspects of metabolic impairment (Netea et al., 1997; Nieto-Vazquez et al., 2008; Pradhan, 2007; You & Nicklas, 2008).

Together with other pro-inflammatory cytokines and various immune cells, TNF-α is an important contributor to the development of atherosclerotic lesions (Popa, Netea, Van Riel, Van Der Meer, & Stalenhoef, 2007). TNF-α and ensuing disease development is initiated through the promotion and expression of adhesion molecules on endothelial cells, the recruitment and activation of inflammatory cells, and the initiation of the inflammatory cascade inside the arterial wall (Popa et al., 2007). In vitro studies have shown that TNF-α increases activation of endothelial smooth muscle NF-κB, which induces the expression of vascular adhesion molecules and cytokines, resulting in inflammatory and foam cell accumulation (Ortego et al., 1999). Inflammation and foam cell accumulation within vasculature results in augmented thrombosis, artherosclerosis and the associated development
of CVD (Hansson & Libby, 2006). Moreover, in 272 participant’s TNF-α concentrations from plasma samples were obtained after an initial MI. TNF-α concentrations were significantly higher among those who suffered a recurrent MI ($\geq 3.74 \text{ pg ml}^{-1}$) compared to those who remained free from reoccurrence ($\leq 3.06 \text{ pg ml}^{-1}$) (Ridker et al., 2000a). During a chronic inflammatory state, TNF-α increases lipolysis without enhancing skeletal muscle fat metabolism (Pradhan, 2007). As such, the chronic inflammatory response initiated by TNF-α promotes an increase of lipids in atherosclerotic plaque; overall, increasing the risk of developing associated chronic diseases such as T2DM and CVD (Netea et al., 1997; Popa et al., 2007; Ridker et al., 2000a).

2.2.3: IL-6

A downstream target of the NF-κB activation and TNF-α expression is IL-6, which represents as an element of a feed forward mechanism of systemic inflammation (De Luca & Olefsky, 2008; Yudkin, Kumari, Humphries, & Mohamed-Ali, 2000). Indeed, IL-6 is an important adipocyte signalling molecule released from visceral and subcutaneous fat stores, which is suggested to represent an association between the level of abdominal adipose tissue and extent of insulin resistance (Fried, Bunkin, & Greenberg, 1998; Park, Park, & Yu, 2005; Van Leuven et al., 2008). Furthermore, this potential relationship between levels of plasma IL-6 and adipose tissue is further extended to anthropometric measurements, such as BMI and WHR (Hu et al., 2004; Meigs et al., 2006). The continuation of insulin resistance and a pro-inflammatory state, expressed through excess adipose tissue and increased IL-6 values, contributes to poor lipid and glucose metabolism, overall representing IL-6 as a clinical biomarker with predictive capacity for the future development of T2DM and CVD (Luc et al., 2003; Pradhan et al., 2001; Ridker et al., 2000b; Sesso, Wang, Buring, Ridker, & Gaziano, 2007; Spranger et al., 2003). For example, a cohort of women (n=27,628) free from clinically
diagnosed chronic diseases (i.e. T2DM, CVD and cancer) at baseline reported that IL-6 concentrations were significantly higher among those who developed diabetes when compared to comparative controls (non-disease state in follow up). The relative risk profiles associated with developing T2DM were placed in quartile ranges. These ranges reported in pg.mL⁻¹ as median (interquartile range) include; Q1, 0.968 (<0.909), Q2, 1.133 (0.91-1.382), Q3, 1.646 (1.383-2.050), Q4, 2.709 (>2.050), with a relative risk for increasing quartiles of IL-6 at 1.0, 2.5, 4.1 and 7.5, respectively (p<0.001 for linear trend). These data show that the relative risk for future diabetes increased 28% per quartile increase in basal IL-6, and supports the possible role for inflammation in predicting the development of T2DM (Pradhan et al., 2001).

Plasma IL-6 concentration also shows a similar linear trend with the relative risk of future MI (Ridker et al., 2000b). Across 14,916 apparently healthy men, those who had a MI reported higher IL-6, with the relative risk increasing with increasing quartiles of baseline IL-6 concentrations (p<0.001 for linear trend). Quartiles ranges (reported in pg.ml⁻¹) were established (Q1: <1.04, Q2: 1.04 – 1.46, Q3: 1.47 – 2.28, and Q4: >2.28; p<0.001 for linear trend) with men at the highest quartile at baseline having a relative risk of 2.3 times higher than those in the lowest quartile (95% CI 1.3 to 4.3). Consequently, each quartile increase in baseline plasma concentrations of IL-6 was significantly associated with 38% increase in risk of a future MI (95% CI 15% to 66%) (Ridker et al., 2000b). The current understanding of the roles of IL-6 and TNF-α in the context of obesity related insulin resistance and CVD is still somewhat ambiguous. However, there is strong enough evidence to support IL-6 as a predictive value for cardiovascular ischemic events, insulin resistance and the future risk of CVD and the development of T2DM. Thus, the reduction of chronic IL-6 concentrations remains a potentially important target for the prevention of inflammation-induced insulin
resistance and atherosclerotic development (Luc et al., 2003; Pradhan et al., 2001; Ridker et al., 2000b; Sesso et al., 2007; Spranger et al., 2003)

2.2.4: CRP

CRP is synthesised in the liver following the stimulation of IL-6, IL-1 and TNF-α (Calabro, Chang, Willerson, & Yeh, 2005; Moshage et al., 1988; Park et al., 2005; Verma et al., 2002). Although not to the same extent CRP can also be produced in atherosclerotic lesions, kidneys, neurons and alveolar macrophages (Calabro et al., 2005). Moreover, through the examination of human aortic endothelial cells, the most potent agonist for CRP production is the combined elevated presence of IL-1 and IL-6 cytokines (Venugopal, Devaraj, & Jialal, 2005). Additionally, adipose tissue secretes adipocytokines that include IL-6, TNF-α, leptin and adiponectin, all of which indicate a major association between obesity and the development of metabolic and vascular diseases (Calabro et al., 2005; Trayhurn & Wood, 2005). Individuals with excess adipose tissue have high circulating concentrations of inflammatory markers responsible for the hepatic and extra hepatic production of CRP, overall indicating the level of systemic distress and an individual’s level of potential disease development (Calabro et al., 2005).

CRP is a common and easily measured inflammatory marker for which clinical cut-off values have been recommended, and prospectively associated with an increased risk of hypertension and the development of chronic diseases (Sesso et al., 2007). A scientific statement was released in 2003 and specified the relative risk categories and mean CRP concentrations to be classified as, low (<1 mg.L⁻¹), average (1.0 to 3.0 mg.L⁻¹) and high risk (>3.0 mg.L⁻¹) (Pearson, et al., 2003). In particular, a cohort of women (n=27,628) free from clinically diagnosed chronic diseases (i.e. T2DM, CVD and cancer) at baseline reported that in addition
to IL-6, CRP concentrations were significantly higher among those who developed diabetes when compared to control. The relative risk profiles associated with developing T2DM were placed in quartile ranges. These ranges are reported as median (interquartile range), mg L\(^{-1}\) and include; Q1, 0.5 (<1.0), Q2, 1.7 (1.0-2.6), Q3, 4.35 (2.7-6.1), Q4, 9.30 (>6.1), with a relative risk for increasing quartiles of CRP at 1.0, 2.2, 8.7 and 15.7, respectively (p<0.001 for linear trend). These data suggest that the relative risk for future diabetes increased 64% per quartile increase in CRP and further supports the possible role for inflammation in predicting the development of T2DM (Pradhan et al., 2001).

In the non-diabetic population, insulin resistance and obesity are important determinants for the increase in inflammatory state (Kahn, Hull, & Utzschneider, 2006). Similarly, the concentrations of inflammatory biomarkers are inversely associated with fasting glucose levels, diminished insulin action, obesity and metabolic syndrome (Bartels et al., 2007; Trayhurn & Wood, 2005). Pradhan et al. (2001) suggest CRP and T2DM may be initiated through the body’s innate immune system and manifest from an ongoing cytokine mediated acute phase response. Moreover, Pradhan (2007) demonstrated that CVD prevalence increased according to risk group from no diabetes or metabolic syndrome, to metabolic syndrome and diagnosed diabetes. In these participant groups an increasing CRP concentration was associated with prevalence of CVD, with both high levels of CRP (>3 mg L\(^{-1}\)) and diabetes had nearly an 8-fold higher prevalence of CVD (OR, 7.73; 95% CI, 3.99 – 14.95) when compared to those with low CRP concentrations and no diabetes (Pradhan, 2007). Collectively, these studies demonstrate that CRP is a valuable clinical bio-marker that signifies the chronic systemic inflammatory state and can be utilised for the risk-stratification associated with the onset of CVD or T2DM (Alberti et al., 2006; Pradhan, 2007).
2.2.5: IL-1

Insulin resistance is characterised by the overcompensation of insulin secretion to maintain glucose disposal. Eventually, the failure to adequately produce insulin (characterised by impaired β-cells function) for maintaining glucose metabolism leads to the onset and diagnosis of T2DM (Alberti et al., 2006; Kitabchi et al., 2013). IL-1β is a pro-inflammatory cytokine implicated as an effector molecule of inflammatory β-cell destruction through apoptosis (Larsen et al., 2007). β-cells producing IL-1β are evident in type 2 diabetics, with high glucose levels increasing β-cell production and the release of IL-1β, which further initiates functional impairment and apoptosis of the β-cells (Larsen et al., 2007). Similarly, the serum concentration of the anti-inflammatory cytokine IL-1ra is elevated in obese and pre-diabetic populations, with an accelerated increase observed before the onset of T2DM (Arend, 2002). The expression of IL-1ra is induced by IL-1β and reflects the response to counterbalance increased activity of IL-1β (Arend, 2002; Donath & Shoelson, 2011). Furthermore, blocking IL-1 activity in those with diagnosed T2DM for 13 weeks resulted in improved glycaemic control (as determined from haemoglobin A1c, HbA1c), C-peptide, IL-6 and CRP concentrations and the ratio of pro-insulin to insulin (Donath & Shoelson, 2011; Larsen et al., 2007). These findings suggest that IL-1β has a role in the pathogenesis of T2DM and that therapeutic interventions aimed at reducing IL-1β concentrations may assist in improving the overall inflammatory state, β-cell functioning, and glycaemic control for the prevention of T2DM.

Of note, IL-1ra is an agonist to the expression of IL-1β and protects human β-cells from glucose induced functional impairment and apoptosis, initiated by IL-1β (Arend, 2002; Hotamisligil, 2006). Accordingly, IL-1ra has anti-inflammatory properties that are important in maintaining a balance between IL-1ra and IL-1β for overall β-cell function and glycaemic
control (Arend, Malyak, Guthridge, & Gabay, 1998; Wellen & Hotamisligil, 2005). IL-1ra is expressed in the pancreas of non-diabetics but is decreased in the islets of patients with T2DM, which enhances the susceptibility of the β-cells to release IL-1β (Arend, 2002; Arend et al., 1998; Donath & Shoelson, 2011). Collectively, the IL-1β system is an integral part of the response to metabolic disturbances and the antagonism through increased IL-1ra concentrations has therapeutic potential (Arend, 2002; Donath & Shoelson, 2011).

2.2.6: Leptin

Leptin is a peptide hormone that is predominately produced in white adipose tissue and assists in regulating neuroendocrine function, energy homeostasis, haematopoiesis and angiogenesis (Meier & Gressner, 2004; Otero et al., 2005). Furthermore, leptin is also considered as a pro-inflammatory cytokine that functions to control appetite and immediate immune-related diseases and inflammatory processes (La Cava & Matarese, 2004; Ropelle et al., 2010). Leptin concentration reflects the amount of energy stored in adipose tissue and is proportional to overall adipose mass and more recently to muscle mass, where it can also be produced (Carson, Livingstone, Pourshahidi, Mccrorie, & Wallace, 2012). More commonly known is the role of elevated leptin in the obese state, where it is thought to contribute to insulin resistance and is considered to be one of the links between obesity, insulin resistance, and atherosclerosis (Berg & Scherer, 2005; Silha et al., 2003). This is further supported by the strong correlations reported between the level of insulin resistance and leptin concentrations (Silha et al., 2003). Moreover, the increase in leptin during states of inflammation strongly suggests that leptin is a part of the cytokine network that is increased with other pro-inflammatory cytokines, such as TNF-α IL-1β IL-6 and CRP (Fernández-Riejos et al., 2010; Meier et al., 2002). While the role of leptin in inflammation is not completely understood, states of hyperleptinemia have shown to be induced by inflammatory
signals, with leptin concentrations showing positive associations with CRP and TNF-α, and a negative correlation with IL-1ra (Fernández-Riejos et al., 2010; Meier et al., 2002). Taken collectively, leptin is a pleiotropic hormone/cytokines that regulates food intake and basal metabolism as a hormone, and as a cytokine, can affect the secretion of IL-1β, TNF-α, IL-6 and IL-1ra (Fernández-Riejos et al., 2010; La Cava & Matarese, 2004; Meier et al., 2002). As such, the over expression of leptin seems detrimental to insulin sensitivity and thus the ensuing onset of T2DM, with a decrease in leptin potentially stimulating therapeutic benefits for overall glucose regulation (La Cava & Matarese, 2004; Meier et al., 2002).

2.2.7: Adiponectin and IL-10

Adiponectin is another hormone secreted by adipocytes which stimulates an increase in the anti-inflammatory cytokines IL-10 and IL-1ra in monocytes and macrophages, while inhibiting systemic levels of IL-6, TNF-α and CRP (Bouassida et al., 2010; Ouchi et al., 2003; Ouchi et al., 2011). Furthermore, the stimulation of anti-inflammatory cytokine IL-10 has also shown to have suppressive pro-inflammatory effects when IL-10 is injected into humans and suppresses the production of pro-inflammatory cytokines TNF-α and IL-1β (Chernoff et al., 1995). Accordingly, the expression of adiponectin and IL-10 can protect against increased pro-inflammatory circulation and in turn metabolic and cardiovascular disorders. As evidence, these anti-inflammatory markers are decreased in plasma and adipose tissue in obese, compared to lean individuals (Carson et al., 2012; Ouchi et al., 2011). Additionally, patients with high concentrations of adiponectin have a reduced 6-year risk of a MI compared to case controls; this relationship still persists after controlling for family history, BMI, hypertension, HbA1c and CRP (Pischon et al., 2004). Taken collectively, a pro-inflammatory state inhibits the production of adiponectin and IL-10, with low levels increasing insulin resistance and risk of CVD, and thus generating a self-sustaining
inflammatory loop (Fantuzzi, 2008). Accordingly, methods for increasing the chronic secretion of adiponectin and IL-10 may also represent a decrease in the pro-inflammatory state (over expression of leptin, IL-6, TNF-α, IL-1β and CRP), which may further show direct improvements in glycemic control and improvement in the balance between pro- and anti-inflammatory mediators.

2.2.8: Summary of chronic systemic inflammation

Chronic systemic inflammation is an important risk factor for several clinical diseases such as CVD and T2DM. Findings from cross-sectional investigations support an inverse relationship between physical activity or aerobic capacity and levels of chronic inflammation in men, women, and children across healthy and diseased participants (Aronson et al., 2004a; Aronson et al., 2004b; Church et al., 2002; Mohamed-Ali et al., 1997; Panagiotakos et al., 2005; Pischon et al., 2003; Roytblat et al., 2000; Visser et al., 1999). Furthermore, Panagiotakos et al. (2005) assessed the association between weekly exercise patterns and inflammatory marker status, taking into account frequency, duration, and intensity data. This particular study highlighted that in comparison to sedentary participants and after adjustment for gender, age, smoking habits, BMI, total cholesterol, glucose, and blood pressure, participants devoted to high physical activity (>7kcal/min expended) reported 19%, 29% and 32% lower concentrations of white blood cells (WBC), CRP and IL-6, respectively (P<0.05) (Panagiotakos et al., 2005). Aronson et al. (2004a) supports such findings through the collection of venous samples for the analysis of CRP from 1,640 participants completing a Bruce treadmill protocol, with results expressed as maximal metabolic equivalents. The results of this study indicated a strong inverse trend towards decreasing baseline CRP values and increasing fitness quartiles in participants without metabolic abnormalities, one or two metabolic abnormalities and participants with metabolic syndrome. Results indicated that
CRP concentrations decreased 0.042 mg·L⁻¹ for each metabolic equivalent gained during the protocol. Collectively, these respective studies demonstrate exercise and physical activity are both capable of inducing a decrease in pro-inflammatory cytokines and subsequently a reduction in risk associated with the onset of chronic disease development.

Accordingly, there is increasing interest in the application of exercise interventions to reduce the chronic inflammatory state and disease risk (Beavers et al., 2010; Donges et al., 2010; Lavie et al., 2011; Nicklas et al., 2005). A primary prevention strategy of CVD and T2DM is increasing levels of physical activity to promote changes in body composition and accordingly restore the balance between pro- and anti-inflammatory mediators (Arend, 2002; Hotamisligil, 2006; Ouchi et al., 2011). Finally, the repercussions of an improved inflammatory state have direct consequences on glycaemic control and insulin signalling, which collectively form as risk-factors associated with the development of metabolic and cardiovascular abnormalities (Arend, 2002; Hotamisligil, 2006; Ouchi et al., 2011).

2.2.9: Inflammation and glucose regulation

Defects in insulin signalling, alterations in expression of adipokines and cytokines, dyslipidemia, intracellular stress and the activation of inflammatory pathways have been implicated as important mechanisms contributing to insulin resistance and atherosclerosis (Hotamisligil, 2006; Ouchi et al., 2011; Wellen & Hotamisligil, 2005). It is the interaction of these factors, combined with genetic predisposition and environmental influences that result in metabolic complications predisposing the development of T2DM and CVD (Hotamisligil, 2006; Ouchi et al., 2011; Pradhan et al., 2001; Ridker et al., 2000a; Sesso et al., 2007). Additionally, increased lipid deposition in adipocytes leads to the production of pro-inflammatory cytokines, including TNF-α, IL-6 and IL-1β, which further activates the NF-κB
pathway through a feed-forward mechanism (Shoelson et al., 2006). Thus, low concentrations of TNF-α result in improved insulin sensitivity and glucose homeostasis, confirming inflammation to be consequential in regulating insulin action (Hotamisligil, 2003, 2006; Netea et al., 1997; Plomgaard et al., 2005). Moreover, an increase in basal concentrations of IL-6 and TNF-α, have been shown to induce insulin resistance by decreasing skeletal muscle insulin signalling, which in turn suppresses glucose transporter 4 (GLUT4) expression/translocation and reduces the efficiency of plasma glucose disposal (Cai et al., 2005; Egan & Zierath, 2013; Hotamisligil, 2003; Pedersen, Febbraio, & Mooney, 2007). Conversely, studies have reported that neither baseline concentrations of CRP or IL-6 correlate with changes in HbA1c, suggesting that reduced systemic inflammation did not play an important part in improved insulin secretion (Larsen et al., 2007). Despite these conflicting results it is suggested that chronic activation of the NF-κB pathway within adipose tissue impairs glucose control through the deregulation of GLUT4 and the expression of inflammatory cytokines; assisting in the overall development of a chronic diseased state (Cai et al., 2005; Hotamisligil, 2003, 2006; Shoelson et al., 2006).

2.3: Role of systemic and skeletal muscle glucose regulation and mitochondrial dysfunction in the development of chronic disease

2.3.1: Overview

Insulin resistance can be defined as decreased responsiveness of peripheral tissues to insulin action (Kitabchi et al., 2005; Lindholm, 2007; Romijn et al., 1993). Specifically, skeletal muscle utilises both glucose and free fatty acids (FFA) as fuel sources for energy production and becomes immune or less responsive to the action of insulin binding (Kitabchi et al., 2005; Lindholm, 2007; Romijn et al., 1993). Plasma insulin concentration is the principal
factor that restrains lipolysis in adipocytes, whilst stimulating glucose uptake in skeletal muscle (Abdul-Ghani & Defronzo, 2010; Thiebaud et al., 1982). During a fasting state, muscle glucose uptake is low and the plasma FFA concentration is elevated, serving as the principle energy source in skeletal muscle, while the brain exclusively utilises glucose (Thiebaud et al., 1982). Following glucose ingestion, the increase in plasma glucose concentration stimulates insulin secretion from the β-cells. The resultant hyperinsulinaemia suppresses lipolysis, leading to decreases FFA concentration and subsequent decrease in the rate of lipid oxidation (Holloszy & Coyle, 1984). Simultaneously, insulin stimulates glucose uptake in the muscle, and the increase in glucose flux, together with the activation of key enzymes in glucose metabolism by insulin leads a marked increase in muscle glucose oxidation (Abdul-Ghani & Defronzo, 2010; Defronzo, Ferrannini, Sato, Felig, & Wahren, 1981a; Defronzo et al., 1989; Holloszy & Coyle, 1984). After glucose is transported into myocytes via the GLUT4 transporter, it is immediately phosphorylated by hexokinase and converted to, and stored as glycogen, or enters the glycolytic pathways for oxidation (Holloszy & Coyle, 1984). At low plasma insulin concentrations, for example, in a fasted state, glycogen synthase and glucose oxidation contribute equally to glucose disposal. However, with increasing plasma insulin concentration, glycogen synthase is activated by insulin and glucose storage is predominate; accounting for ~60-70% of glucose disposal (Thiebaud et al., 1982).

Skeletal muscle is the major site for glucose disposal in lean healthy glucose tolerant individuals (Defronzo et al., 1981b; Holloszy & Coyle, 1984; Kristiansen et al., 2000). Following a meal, approximately one third of ingested glucose is absorbed by the liver and the remaining by peripheral tissues, primarily by skeletal muscle via insulin dependent mechanisms (Cherrington, 1999; Zierath et al., 2000). The postprandial hyperglycemia
stimulates insulin secretion from the pancreas and the rise in plasma insulin concentrations stimulates glucose uptake and subsequent metabolism in skeletal muscle (Cherrington, 1999; Zierath et al., 2000). In a state of insulin resistance (i.e. T2DM and obesity) insulin stimulated glucose disposal in skeletal muscle is markedly impaired (Defronzo et al., 1989; Zierath et al., 2000). The decreased insulin-stimulated glucose uptake is due to impaired insulin signalling and post-receptor intracellular defects, including impaired glucose transport and phosphorylation, and reduced glucose oxidation and glycogen synthesis (Defronzo et al., 1989; Eckardt et al., 2011; Pradhan, 2007; Zierath et al., 2000). The exact mechanism that leads to the development of insulin resistance in skeletal muscle is not fully understood. It is proposed that a combination of factors associated with systemic inflammation and defects in mitochondrial functioning collectively contribute to reduced insulin release, insulin sensitivity and signalling within the muscle (Egan & Zierath, 2013; Finck & Kelly, 2006; Kim et al., 2008; Lira et al., 2010). Such developments lead to increased systemic concentrations of glucose and insulin in a fasting and fed state, precipitating the onset of insulin resistance and associated development of T2DM (Egan & Zierath, 2013; Finck & Kelly, 2006; Kim et al., 2008; Lira et al., 2010). However to understand the development of insulin insensitivity and poor glucose regulation, a further understanding of the dynamic markers of glucose regulation require discussion. The severity of metabolic abnormalities can fluctuate, and the degree of hyperglycemia reflects the severity of the underlying metabolic process (Kitabchi et al., 2013). Accordingly, to obtain any clinical relevance regarding these underlying metabolic processes a clinical assessment of glucose regulation should be obtained.
2.3.2: Clinical assessments of glucose regulation

Metabolic homeostasis is largely controlled by the balance between the anabolic hormone insulin and the catabolic hormone glucagon, both produced by the pancreatic islets of Langerhans (Cherrington, 1999; Vilsbøll, Krarup, Deacon, Madsbad, & Holst, 2001). Insulin secretion from islet β-cells is regulated by a number of factors that occur with the ingestion of carbohydrate containing meals. Insulin stimulates both glucose oxidation and non-oxidative glucose disposal, which largely consists of storage of muscle glycogen (Yki-Järvinen, Mott, Young, Stone, & Bogardus, 1987). Accordingly, the stimulation of glucose disposal is one of the most characteristic actions of insulin, with muscle tissue playing a dominating role in glucose disposal and storage by insulin (Yki-Järvinen et al., 1987). Metabolic syndrome (hyperglycemia, dyslipidemia, and hypertension) contributes to the development and progression of CVD, particularly in men aged ≥45 and women aged ≥55 y (Bartels et al., 2007; Lorenzo, Williams, Hunt, & Haffner, 2007). Genetic susceptibility and environmental factors contribute to poor nutrition, obesity, systemic inflammation and physical inactivity, all of which collectively influence chronic disease development (Bartels et al., 2007; Lorenzo et al., 2007). Impaired insulin secretion (hypoinsulinism) and/or signalling leads to insufficient cellular glucose uptake and a hyperglycemic state (Dandona et al., 2004). In clinical settings, a common method to determine the state of glucose regulation and diagnosis of metabolic disease is via measures of HbA1c (%A1c), fasting glucose and insulin, estimated insulin resistance (HOMA-IR), estimated insulin sensitivity (Matsuda ISI) and/or an OGTT (Alberti et al., 2006; Matsuda & DeFronzo, 1999; Matthews et al., 1985).

It is recognised that an intermediate group of individuals whose glucose levels do not meet the criteria for diabetes, yet are higher than ‘normal’ (Fasting plasma glucose 3.9–5.6 mmolL⁻¹) are generally defined as having IFG (fasting plasma concentrations of 6.1–7.0
mmolL^{-1}), without IGT. Additionally, IGT is defined as having a fasting glucose concentration of <7.0 mmolL^{-1} and a 2 h post glucose load (75 g OGTT) of 7.8–11.1 mmolL^{-1} (Tabak, Herder, Rathmann, Brunner, & Kivimäki, 2012). These indices are defined by the World Health Organization, although and the American Diabetes Association (ADA) applies these same thresholds for IGT but uses lower cut-off points for IFG (5.6–6.9 mmolL^{-1}); in addition to the inclusion of HbA1c indices as a new category for high diabetes risk (Kitabchi et al., 2013). These individuals are referred to as pre-diabetic and have a relatively high risk of developing T2DM. Rather than a clinical diagnosis, categorising these groups as having IFG and/or IGT should be viewed as risk-factors for developing multiple diseases such as T2DM and/or CVD (Kitabchi et al., 2005, 2013).

HbA1c is more commonly used to identify those at high risk for the development of the disease (i.e. those who are obese and/or inactive and those with a family history of the disease) (Zhang et al., 2010). Specifically, prospective studies indicate that those within an A1c range of 5.5-6.0% have a 5 y cumulative incidence of diabetes that ranges from 9-25% (Edelman, Olsen, Dudley, Harris, & Oddone, 2004; Sato et al., 2009; Zhang et al., 2010). Comparatively, those in ranges between 6.0-6.5% A1c had a 5-year risk of developing diabetes between 25-50%, including a 20-fold higher relative risk compared with those reporting an A1c of 5.0% (Zhang et al., 2010). Indeed, linear regression of this data indicates that among the non-diabetic cohort fasting plasma glucose of 6.1 and 5.6 mmolL^{-1} corresponds with 5.6% and 5.4% A1c, respectively (Kitabchi et al., 2013). However, it should be noted that a range of 6.0-6.5% has previously failed to show associations with IFG and IGT (Edelman et al., 2004; Sato et al., 2009; Zhang et al., 2010). Regardless, diabetes prevention programs have shown to be effective in participants above and below 5.9% A1c, with a range of 5.5-6.0% A1c highlighted as ideal to initiate preventive approaches (Knowler
et al., 2002; Lindström et al., 2006). Furthermore, the relationship of HbA1c and CVD is noted by reports showing that disease-free participants with concentrations <5% A1c had the lowest chance for the future development CVD (Khaw et al., 2004). Indeed, every 1% increase in HbA1c past 6%, increases the relative risk for death of any cause by 1.24 (95% CI, 1.14-1.34; p<0.001) in men and 1.28 (95% CI, 1.06-1.32; p<0.001) in women (Khaw et al., 2004). While both fasting plasma glucose and HbA1c are independently associated with the risk of T2DM and CVD, the combination of these variables is reported to be a more accurate predictor of disease development, and thus stand as important clinical predictors of the diseased state (Sato et al., 2009).

Impaired insulin secretion and action are the two main pathophysiological disturbances leading to abnormal glucose tolerance (Kuroe et al., 2003). To retain normal glucose concentrations in response to a glucose load, increased insulin secretion is required to compensate for decreased insulin sensitivity (Inzucchi, 2012). Accordingly, individuals may have normal glucose concentrations at rest and in response to a glucose load, even though insulin resistance and hyperinsulinaemia may be evident (Tabak et al., 2012). To support this compensatory role of insulin secretion to maintain normal glucose metabolism, a longitudinal study of glucose responses constructed a 13 year trajectory of fasting and post load (OGTT) blood glucose, insulin sensitivity and insulin secretion until diabetes diagnosis in a metabolically healthy middle-aged cohort. This study reports those who developed diabetes, initially had lower insulin sensitivity (HOMA: incident diabetes, 103.4%; non-diabetics, 145.1%), and higher fasting (incident diabetes, 12.17 µIU.mL\(^{-1}\); non-diabetics, 7.83 µIU.mL\(^{-1}\)) and post glucose load insulin (incident diabetes, 78.83 µIU.mL\(^{-1}\); non-diabetics, 43.17 µIU.mL\(^{-1}\)) concentrations (Tabák et al., 2009). Furthermore, HOMA insulin sensitivity showed a steep decrease within the last 5 y prior to diagnosis, and during this time insulin
secretion increased to compensate for decreased insulin sensitivity, without major changes in fasted glucose concentrations (Tabák et al., 2009). Of note, this collection of findings seems to be consistent across a range of literature (Lyssenko et al., 2005; Tabak et al., 2012; Tripathy et al., 2000). Even more pertinent, when insulin sensitivity is decreased and insulin secretion is unable to maintain normal blood glucose concentrations through impaired β-cell function, then pre-diabetes or diabetes becomes evident (Tripathy et al., 2000). Collectively, it is the contribution of all variables (fasting and post load insulin and glucose concentrations, insulin sensitivity and HbA1c) that allows for the clinical analysis and classification of an individual’s metabolic state. Accordingly, a comprehensive assessment should be undertaken including all variables to identify and quantify systemic glucose regulation. However, these clinical markers represent the façade of glucose regulation, and not the actual mechanisms controlling and regulating glucose movements.

2.3.3: Glucose regulatory mechanisms in skeletal muscle

The aforementioned markers represent a description in the functional use of systemic glucose; however, muscle is a primary site for insulin dependent glucose disposal – particularly from the perspective of exercise (Defronzo et al., 1981b; Richter & Hargreaves, 2013). In particular, insulin sensitive glucose transporter isoform, GLUT4 redistributes from intracellular storage vesicles to the plasma membrane following insulin stimulation (Kennedy et al., 1999; Sano et al., 2003). The physiological significance of this process is the 10 to 40-fold increase in glucose flux into the cell for metabolism (Kennedy et al., 1999; Sano et al., 2003). Insulin rapidly and persistently activates protein kinase protein kinase B (Akt/PKB), with substantial evidence indicating that Akt is important in directing GLUT4 vesicles to the plasma membrane and promoting glucose transport into the cell (Karlsson et al., 2005; Zierath et al., 2000). Accordingly, there is consistent circumstantial evidence that correlates
changes in Akt activity with changes in glucose transport (Karlsson et al., 2005; Zierath et al., 2000). For example, antagonising insulin-activated glucose transport and GLUT4 translocation in adipocytes and myotubes correlates with a 60% decrease in insulin-stimulated Akt activity (Karlsson et al., 2005; Whiteman, Cho, & Birnbaum, 2002). This decrease in insulin stimulated activity also impairs ability of insulin to suppress hepatic glucose production and to stimulate peripheral glucose clearance as well as compensatory hyperinsulinaemia (Roden et al., 1999; Vind et al., 2012). Accordingly, insulin resistance in skeletal muscle represents as an early defect occurring in the pathogenesis of T2DM (Roden et al., 1999; Roden et al., 1996; Vind et al., 2012).

Akt is a key signalling protein responsible for glucose transport (Whiteman et al., 2002). Upon full activation of Akt, the phosphorylation of serine 473 and threonine 308 are necessary for subsequent stimulation of AS160 phosphorylation for the critical up-regulation of GLUT4 translocation to the plasma membrane (Bruss, Arias, Lienhard, & Cartee, 2005; Sakamoto & Holman, 2008; Whiteman et al., 2002; Wu, Falasca, & Blough, 2011). The expression of Akt is generally reduced in insulin resistant muscle, and increases in Akt protein expression are associated with ameliorating insulin resistance (Karlsson et al., 2005). Accordingly, obese participants with hyperglycaemia have a lower expression of Akt protein, while normalising blood glucose after insulin therapy is able to increase basal Akt expression alongside reductions in BMI (Zierath et al., 2000). However, this finding is not consistent through the literature, and several reports have demonstrated that insulin sensitivity in skeletal muscle can be modulated despite normal phosphorylation of Akt (Kruszynska et al., 2002; Storgaard et al., 2004; Treebak et al., 2009; Wu et al., 2011). Furthermore, alterations in GLUT4 expression and function have been associated with insulin resistant states such as obesity and T2DM (Després & Lemieux, 2006). In particular, deleting the GLUT4 gene has
shown to consistently predispose metabolic abnormalities and T2DM in rodent models (Nandi, Kitamura, Kahn, & Accili, 2004). Conversely, an increase in glucose disposal is evident when gene expression and protein content of GLUT4 is up-regulated through muscle contraction (Goodyear & Kahn, 1998; Richter & Hargreaves, 2013). Comparatively, GLUT4 nuclear abundance and mRNA expression are equivalent in the muscle from diabetic and lean normo-glycemic participants (Garvey, Maianu, Hancock, Golichowski, & Baron, 1992; Pedersen et al., 1990). However, this is not a consistent finding, with more recent studies identifying decreases in GLUT4 expression and/or translocation in response to states of impaired fasting glucose, glucose tolerance and/or T2DM (Garvey et al., 1998; Kennedy et al., 1999; Richter & Hargreaves, 2013). Collectively, the literature present inconsistent findings, although it does appears that GLUT4 and Akt abundance and activity may not be the sole mechanism necessary for the augmentation of glucose metabolism via skeletal musculature.

2.3.4: Insulin sensitivity and the role of mitochondria

Insulin stimulates glucose uptake in peripheral tissues, such as skeletal muscle, and suppresses hepatic glucose production (Cherrington, 1999). Insulin sensitivity is generally believed to decline with old age, although accumulating evidence supports the notion that physical inactivity and adiposity, and not chronological age per se, are strong predictors of the decline in insulin sensitivity with age (Choi et al., 2008; Petersen, Dufour, Befroy, Garcia, & Shulman, 2004). Metabolic disturbances such as progressive declines in whole body peak oxygen consumption (VO_{2peak}) have been observed, even after correcting for lean mass (Lanza & Sreekumaran Nair, 2010; Rogers, Hagberg, Martin, Ehsani, & Holloszy, 1990; Short et al., 2005). At a cellular level, there has been increased focus in an attempt to understand the changes in the mitochondrion with age, as a mechanism to understand
alterations to cellular control of energy metabolism (Lanza & Sreekumaran Nair, 2010). Accordingly, the mitochondria have been implicated in the age-related decline in insulin sensitivity with this notion largely driven by correlative evidence in obese, type 2 diabetics and elderly participants (Kim et al., 2008). Interestingly, a decline of insulin sensitivity and mitochondrial oxidative capacity in young and older men have shown to be driven more by physical activity and adiposity, not chronological age (Lanza & Sreekumaran Nair, 2010). Alongside these findings, further literature has emerged indicating insulin as a regulator of mitochondrial biogenesis (Petersen et al., 2004; Stump, Short, Bigelow, Schimke, & Nair, 2003). Although the literature is inconsistent, it may be reasonable to assume that with insulin resistant states the mitochondrial signalling pathways and protein expression may also be blunted. Skeletal muscle mitochondria from T2DM obese subjects exhibit reduced mitochondria size, whilst endurance exercise overcomes these attenuations in mitochondria function as it improves insulin sensitivity and increases fat oxidisation (Russell, Hesselink, Lo, & Schrauwen, 2005; Wang, Hiatt, Barstow, & Brass, 1999). Given the role of mitochondria in regulating cellular glucose uptake and providing energy balance, positive mitochondrial adaptations to exercise may provide a method to improve glucose tolerance (Hoppeler & Fluck, 2003). As such, the preservation of aerobic capacity and skeletal muscle strength through exercise training can ameliorate metabolic abnormalities, potentially through the extensive molecular remodeling of the skeletal muscle mitochondria (Egan & Zierath, 2013; Hoppeler & Fluck, 2003).

The over expression of peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α) has demonstrated to activate gene regulatory programs that drive increased capacity for cellular energy production (Lin, Puigserver, Donovan, Tarr, & Spiegelman, 2002; St-Pierre et al., 2003; Wu et al., 1999). PGC-1α equips the cell to meet energy demands
of a changing milieu, including augmentation of mitochondrial biogenesis, cellular respiration rates, energy substrate uptake and utilisation (Finck & Kelly, 2006; Wu et al., 1999). All of which have direct implications with metabolic processes, insulin sensitivity and gluconeogenesis (Russell et al., 2005). PGC-1α docks on and co-activates transcription factors that regulate expression of nuclear respiratory factors (NRF)-1 and NRF-2, which are nuclear genes that encode mitochondrial proteins and also of the nuclear gene that encodes mitochondrial DNA transcription factor A (Tfam) (Finck & Kelly, 2006; Fisher, Lisowsky, Parisi, & Clayton, 1992; Wu et al., 1999). Thus, PGC-1α regulates the coordinated expression of the respiratory chain and the biogenesis of mitochondria. Conversely, mitochondrial dysfunction can be demonstrated by reduced activity and content of key enzymes and proteins involved in oxidative metabolism pathways and the electron transport chain (Russell et al., 2005). Accordingly, mitochondrial dysfunction in diabetic and obese subjects is suggested to have a crucial influence in the pathogenesis of insulin resistance and in turn plasma and skeletal muscle glucose regulation (Coletta & Mandarino, 2011; Russell et al., 2005) Consequently, understanding the response of PGC-1α to exercise, alongside glucose and insulin sensitivity, may highlight the mechanisms underlying improved systemic glucose regulation in response to exercise-based interventions.

Furthermore, two regulatory proteins responsible for the activity and expression of PGC-1α are Sirtuin-1 (SIRT1) and protein 53 (p53). SIRT-1 is likely to contribute to the development of insulin resistance through its regulatory effect on adipokines and insulin signalling (Liang, Kume, & Koya, 2009; Tonkin, Villarroya, Puri, & Vinciguerra, 2012). Previous studies have focused on the anti-inflammatory effects of SIRT-1 and the contribution to PGC1-α deacetylation, thus providing direct evidence for SIRT-1 related attenuation of inflammation during insulin resistance (Lin et al., 2012; Yoshizaki et al., 2010). Moreover, the close
relationship between SIRT-1 and PGC1-α might provide some insight into the association between SIRT-1 and mitochondrial function (Gerhart-Hines et al., 2007; Liang et al., 2009; Rodgers, Lerin, Gerhart-Hines, & Puigserver, 2008). In skeletal muscle, SIRT-1 mediated deacetylation of PGC1-α is also required to activate genes associated with mitochondrial fatty acid oxidisation in response to energy demand (Liang et al., 2009). During such activity, the transcriptional activation of PGC1-α depends on SIRT-1 nuclear accumulation (Liang et al., 2009). Collectively, the effects of PGC1-α in activation of GLUT4 gene expression are reflected in the increased ability of myocytes to transport glucose, further suggesting that SIRT1-regulated PGC-1α is an indirect influence on insulin sensitivity (Liang et al., 2009; Michael et al., 2001). Moreover, recent research indicates that the tumor suppressor p53 localises in the mitochondria and is stimulated by cellular stressors, including DNA damage and hypoxia, in addition to mitochondrial biogenesis and respiration (regulates oxidative phosphorylation (OXPHOS) subunit (complex (COX) II) assembly) (Hock & Vousden, 2012; Manoli et al., 2007; Matoba et al., 2006; Park et al., 2009). As such, p53 becomes a potential target and protein of interest when investigating the cause and prevention of chronic diseases where the mitochondria are implicated (Bartlett et al., 2012; Manoli et al., 2007; Saleem, Carter, & Hood, 2013).

Collectively, these links between mitochondrial function and glucose control may assist to explain the beneficial effects of (endurance) exercise, specifically for counteracting T2DM and related metabolic and cardiovascular disorders. Understanding the mechanisms of mitochondrial regulators and how they interact will assist in identifying and improving preventative and therapeutic strategies for metabolic disease (Cantó & Auwerx, 2009). Figure 2.4 provides a schematic overview relating the two main topics of this thesis, glucose regulation and inflammation. Furthermore, this diagram further identifies the importance of
the mitochondrial within these processes of disease development and the overall dictating contribution of physical inactivity and obesity.

Figure 2.4
Schematic overview relating the glucose regulatory and systemic inflammatory topics of this thesis. Furthermore, this diagram further identifies the importance of mitochondrial content and activity within these processes of disease development, which can be directly and indirectly dictated by physical inactivity and obesity.
2.4: An exercise focused approach to prevent the onset of chronic disease

Physical inactivity is reported to promote the development of obesity and is strongly associated with preventable chronic diseases such as T2DM and CVD (Alberti et al., 2006; Laaksonen et al., 2002; Rissanen, Heliövaara, Knekt, Reunanen, & Aromaa, 1991). Alternatively, regular exercise can be an effective primary prevention strategy against chronic disease development that can assist to counter the deterioration of the physiological state, as represented through chronic systemic inflammation and glucose regulation. In turn, improved physical health of the general public can also offset the economic and social repercussions of these diseases across both Indigenous and non-Indigenous Australian populations (Durstine et al., 2012). Indeed, the benefits of exercise for chronic disease prevention have traditionally been associated with systemic adaptations and improvements in clinical risk factors (i.e. fat-mass, muscle mass, inflammatory state, aerobic capacity, lipid profile) associated with glucose regulation and/or insulin sensitivity (Alberti et al., 2006; Durstine et al., 2012; Laaksonen et al., 2002). In part, these clinical benefits have been attributed to metabolic and mitochondrial remodeling within skeletal muscle (Egan & Zierath, 2013).

To date, the majority of research has focused on these respective clinical health benefits in relation to continuous, aerobic-based exercise training, such as cycle ergometry (Egan & Zierath, 2013; Garber et al., 2011). Such exercise stimuli predominately involves lower-body, concentric muscular contractions capable of reducing fat-mass, and improving the chronic inflammatory state, aerobic capacity, glucose regulation and mitochondrial biogenesis (Balducci et al., 2010; Bijker et al., 2002; Donges et al., 2010; Egan & Zierath, 2013; Goodyear & Kahn, 1998; Hawley & Lessard, 2008; Irrcher et al., 2003; Libardi et al., 2012). Accordingly, the ACSM recommend that ‘apparently healthy’ adults engage in moderate-
intensity cardiorespiratory exercise for ≥30 min·d⁻¹ on ≥5 d·wk⁻¹ for a total of ≥150 min·wk⁻¹, vigorous-intensity cardiorespiratory exercise training ≥20 min·d⁻¹ on ≥3 d·wk⁻¹ for a total of ≥75 min·wk⁻¹, or a combination of moderate- and vigorous-intensity exercise (Garber et al., 2011). In particular, a recent ACSM position stand recommends incorporating interval training (varying intensity of fixed intervals over a single exercise bout) as an additional approach for exercise prescription (Garber et al., 2011). Of note, this review reports that interval-based training can result in similar or greater adaptations in aerobic capacity, glucose regulation, mitochondrial activity and pro-inflammatory states, when compared to continuous exercise in healthy adults (Croft et al., 2009; Gormley et al., 2008; Helgerud et al., 2007; Nybo et al., 2010; Whyte et al., 2010). However, it also acknowledges the clear lack of evidence within inactive cohorts, and that further research is required to fully understand the health benefits across different modes of interval specific exercise.

Small-sided games incorporate frequent bouts of high-intensity efforts, separated by low-intensity movements over prolonged durations (Bijker et al., 2002; Campbell et al., 2014). Accordingly, varying the interval characteristics of SSG may consolidate the benefits associated with both interval and continuous training. Furthermore, sports-specific SSG’s is a group training approach that provides an additional method of prescription for those without access to specialised equipment and/or those not attracted to gym-based training (Krustrup et al., 2010c; Neesham & Garnham, 2012; Randers et al., 2012). It is assumed the regularity of this acute stress via exercise training promotes improved chronic adaption in mitochondrial biogenesis, glucose regulation, body composition and systemic inflammation (Gleeson, 2013; Lanza & Sreekumaran Nair, 2010). In turn, such physiological adaptations are suggested to improve clinical risk factors for the overall prevention of chronic disease development (Ishii et al., 1998; Jurca et al., 2005; Krustrup et al., 2013; Unwin et al., 2002).
Further, no literature currently outlines the respective acute demands of SSG within inactive populations compared to traditional cycle ergometry. A greater understanding of these exercise-induced responses would assist in explaining the chronic adaptations as related to systemic inflammation, glucose regulation and mitochondrial biogenesis within Caucasian men. Moreover, despite high rates of chronic disease, obesity and physical inactivity no acute or chronic exercise programs have been assessed within Indigenous Australian men – which is particularly surprising given the exacerbated rates of preventable chronic diseases between ancestral populations mentioned earlier.

### 2.5: Acute and chronic exercise-induced responses for anti- and pro-inflammatory markers

#### 2.5.1: Acute inflammatory responses to exercise

Growing evidence links T2DM and CVD to a state of chronic low-grade-inflammation (Libby, Ridker, & Maseri, 2002; Pradhan, 2007; Pradhan et al., 2001; Ridker, 2003; Ridker et al., 2003). However, depending on the tissue of origin, it is now well known that IL-6 has both anti- and pro-inflammatory properties when released from myocytes and adipocytes, respectively (Pedersen, 2011a; Petersen & Pedersen, 2005). Over the past decade research has sought to determine the source of IL-6 during different acute exercise bouts that may explain the chronic adaptations to exercise and inactivity, respectively (Nybo, Nielsen, Pedersen, Moller, & Secher, 2002; Pedersen, Steensberg, & Schjerling, 2001; Steensberg et al., 2002a). A consensus exists that IL-6 is predominately expressed in contracting skeletal muscle/s and results in increased systemic concentrations of the cytokine following exercise. Such findings are based on experimental models involving one and two legged knee
extension exercises (Keller et al., 2001), catheterisation of the femoral vein and arteries (Steensberg et al., 2002a), and biopsies of skeletal muscle for samples of IL-6 mRNA (Steensberg et al., 2002a). These respective studies concluded that IL-6 is released from contracting limbs, due to the increase in IL-6 transcriptional rate in the nuclei of myocytes.

Accordingly, IL-6 is expressed within myocytes of contracting limbs and accounts for the increase in plasma IL-6 concentration in response to exercise. It is suggested the release of IL-6 from myocytes is regarded as an anti-inflammatory cytokine due to the ensuing stimulation and increased circulating levels of other anti-inflammatory cytokines, including IL-1ra and IL10 (Steensberg, Fischer, Keller, Moller, & Pedersen, 2003). This anti-inflammatory expression in turn suppresses TNF-α production, which provides further evidence of the acute post-exercise anti-inflammatory responses as mediated by muscle derived IL-6. Consequently, the post-exercise increase in IL-6 can in part, protect against TNF-induced insulin resistance (Starkie, Ostrowski, Jauffred, Febbraio, & Pedersen, 2003). Figure 2.5 illustrates the exercise-induced effects of exercise, when compared to a TNF-α induced immunological challenge as evident during sepsis.
The magnitude of the acute inflammatory response tends to be dictated by factors such as, the endurance capacity of the cohort, the muscle mass involved to complete the mechanical work and the intensity and duration of the exercise bout (Mendham, Donges, Liberts, & Duffield, 2011; Pedersen et al., 2003; Steensberg et al., 2003). During exercise there is an inverse relationship between exercise intensity and duration (Steensberg, Toft, Schjerling, Halkjaer-Kristensen, & Pedersen, 2001b). Therefore, the relationship between increased plasma IL-6 and exercise duration may be more amplified if adjusted for exercise intensity (Steensberg et al., 2001).

**Figure 2.5** Comparisons of sepsis-induced versus exercise-induced increases in circulating cytokines. During sepsis or immunological strain TNF-α is followed by an increase in IL-6. In contrast, during exercise, the marked increase in IL-6 is not preceded by elevated TNF-α.

Fischer (2006) suggested that the release of IL-6 into the plasma is highly dependent on the duration of exercise, with duration accounting for more than 50% of IL-6 variation within the plasma. Moreover, a short duration (6 min) maximal rowing ergometer exercise protocol in 8 healthy trained oarsman resulted in significant 3-fold increases of plasma IL-6 concentration (p<0.05) when compared to resting values (Nielsen, Secher, Christensen, & Pedersen, 1996). Conversely, when 8 healthy men completed an all-out (90% VO$_{2\text{max}}$) cycle ergometry bout for 5 min and they showed no change in plasma IL-6 concentration throughout a 24 h recovery period (Brenner et al., 1999). In comparison, a long duration (2 h), moderate intensity (60-65% VO$_{2\text{max}}$) cycle ergometry bout increased IL-6 concentration from 1.14 to 6.06 pg.mL$^{-1}$ at 3 h post-exercise (Brenner et al., 1999). These respective studies suggest that the IL-6 response to a high-intensity, short duration bout may be associated with higher muscle mass recruitment – as observed in rowing when compared to cycle ergometry. However, when the cycle ergometry session compensated for a lower muscle mass requirement and lower intensity with an increase in duration, IL-6 still indicated a substantial increase. As further evidence, a 10-fold increase of plasma IL-6 levels requires exercise to last up to 1.9 h (CI 1.6-2.9 h, p<0.001), while an IL-6 increase of 100-fold requires exercise lasting up to 6 h (CI 4.5-8.1 h, p<0.001) (Pedersen & Febbraio, 2008). Accordingly, when taking into account the plasma IL-6 response being amplified to muscle mass recruitment, intensity and duration the mode of exercise can be modified accordingly (Pedersen & Febbraio, 2008).

Previous research suggests ensuring some extent of muscle damage i.e. including a modality of resistance and/or eccentric muscle contractions, or glycogen depletion are important acute regulators of IL-6 release (Gollnick, Piehl, & Saltin, 1974). Previously, it was considered that the acute IL-6 response was generated specifically as a result of muscle damage (Toft et al.,
2002). Such conclusions led to comparing intensity matched eccentric and concentric exercise protocols which resulted in similar serum IL-6 responses (Willoughby, Vanenk, & Taylor, 2003). In response, these papers concluded that muscle damage is not a major stimulus of the immediate post-exercise increase in IL-6 (Willoughby et al., 2003). More recently, IL-6 has consistently been shown to increase within non-contracting skeletal muscle, with the increase augmented when intramuscular glycogen levels are depleted (Chan, Carey, Watt, & Febbraio, 2004a) or suppressed through carbohydrate ingestion during and after exercise (Keller et al., 2001; Miles, Walker, Conant, Hogan, & Kidd, 2006; Ronsen, Lea, Bahr, & Pedersen, 2002).

Aligned to the above observations on muscle glycogen, previous reports show a more substantial increase in IL-6 in running when compared to cycle ergometry (Pedersen & Febbraio, 2008). These results are not mode specific per se, rather it is the level of muscle mass recruitment and/or glycogen depletion (intensity and duration dependent) within each modality that may be a more suitable description of mechanisms responsible for the acute IL-6 response (Pedersen & Febbraio, 2008). Indeed, the majority of literature has focused on trained and/or younger cohorts with minimal studies specific to inactive/sedentary and/or overweight populations. Accordingly, more research is required specific to this population, especially given the potential exercise-induced effects on chronic systemic inflammation and ensuing relationship to disease development in these populations. Furthermore, the studies specific to inactive populations have applied traditional exercise modes such as walking, cycle ergometry or gym-based resistance exercise modes. In particular, these results show a sub-maximal cycle ergometry to volitional exhaustion in middle-aged, inactive men stimulated and increases in plasma IL-6 by ~81% immediately post-exercise (Gray, Robinson, & Nimmo, 2008). However, 30 min of moderate intensity (50% VO_2max) walking
at 3% incline reported no significant changes in IL-6 up to 168 h post-exercise recovery (Markovitch, Tyrrell, & Thompson, 2008). Furthermore, IL-6 response to strength and cycle ergometry conditions of the same duration is reported to be dictated by intensity rather than exercise mode (Mendham et al., 2011).

An example of the mode-specific responses to exercise focusing on continuous and resistance exercise models has included middle-aged, inactive populations (Mendham et al., 2011). Specifically, duration matched continuous aerobic (cycle ergometry) or resistance (machine weight) exercise of higher and lower intensities (higher intensity, ~80% HR\text{max}; lower intensity, ~60% HR\text{max}) the IL-6 response was dictated by the intensity, rather than the exercise mode (Mendham et al., 2011). Alternatively, high-intensity intermittent cycle ergometry (ten 4-min intervals with 2 min rest periods) showed a significantly greater increase in IL-6 when compared to moderate intensity continuous cycle ergometry. In particular, IL-6 peaked immediately post-exercise in both conditions; however, a greater increase was evident in the intermittent condition compared to continuous cycle ergometry (Leggate, Nowell, Jones, & Nimmo, 2010). In explaining these findings, the differences between conditions of the same modes may be a consequence of higher glycogen utilisation within intermittent exercise when compared to continuous modes. To date, this is the only study directly comparing intermittent and continuous exercise modes in young, trained men, with no studies specific to inactive, overweight populations. Regardless, this previous report indicates the potential for intermittent exercise to stimulate a greater level of glycogen depletion and thus an amplified and/or sustained elevation of IL-6, when compared to continuous exercise.
The modes of exercise consistently reporting the most amplified IL-6 responses include events such as marathons and triathlons; with the observed increase occurring alongside an increase in pro-inflammatory cytokines such as IL-1β and TNF-α (Gleeson, 2007; Neubauer et al., 2008). Specifically, an ultra-endurance ironman triathlon performed by 42 well-trained male triathletes induced a systemic inflammatory response. The race duration was ~10 h 52 min and involved varying modes of exercise. Immediately post-exercise plasma IL-6 had increased dramatically (+104-fold; p<0.001) and despite a sharp decline, IL-6 values remained significantly elevated 1 d (+345%; p<0.001) and 5 d (+79%; p<0.001) after the race. Plasma CRP concentration rose significantly (543%; p<0.001) immediately after the race and had increased by 77-fold (p<0.001) 1 d post-race. Subsequently CRP decreased but remained significantly higher than pre-race values at 5 d (+881%; P<0.001) and 19 d (+38%; p<0.001) after the competition. Consequently, despite differing modes within the triathlon, plasma IL-6 and CRP levels maintained a significant response up to 5 and 19 d post exercise (Neubauer et al., 2008).

Additionally, 10 men completing a marathon showed a 128-fold increase in IL-6 immediately post and a 39-fold increase in IL-1ra 1 h post exercise (Ostrowski, Rohde, Asp, Schjerling, & Pedersen, 1999). Furthermore, TNF-α and IL-1β peaked immediately post-exercise with TNF-α remaining elevated up to 4 h post (Ostrowski et al., 1999). These authors conclude that it is likely the high-intensity and long duration exercise involvement, such as marathons and ultra-endurance events, are required to stimulate immunological strain and immediately elevate CRP, IL-1β and TNF-α within the post-exercise period. Given the pleiotropic activity of IL-6 and its association with both metabolic and the innate immune system, this immunological strain may justify the sustained amplification of IL-6 over a 5 day recovery period from exercise of extreme duration and intensity (Walsh et al., 2011). Although,
running is a weight-bearing exercise involving large muscle groups and is the mode of exercise relating to the most dramatic increases in IL-6 (Fischer, 2006; Pedersen & Febbraio, 2008). These strenuous and long duration training methods are generally not prescribed with sedentary middle-aged cohorts and may explain the lack of literature describing changes in pro-inflammatory markers in response to an acute exercise bout. Overall, the combination of muscle mass recruitment, intensity and duration of an acute exercise bout has shown to individually and collectively determine the magnitude of the exercise induced increase in plasma IL-6 concentrations (Fischer, 2006; Pedersen & Febbraio, 2008). Therefore, exercise involving limited muscle mass, may prove insufficient in obtaining a significant acute IL-6 response (Fischer, 2006; Pedersen & Febbraio, 2008; Steensberg et al., 2000).

Typically following exercise, the active skeletal muscle increases both cellular and circulating levels of IL-6 (Steensberg et al., 2000). With the exception of extreme duration exercise stimulating immunological strain (Neubauer, König, & Wagner, 2008; Walsh et al., 2011) the acute increase in IL-6 is transient and produced independently to pro-inflammatory cytokines (TNF-α and IL-1β) (Gleeson, 2013; Starkie et al., 2003). Moreover, IL-6 has shown to be responsible for an ensuing increase in anti-inflammatory cytokine IL-1ra (agonist to IL-1β), hepatic synthesis of CRP, suppression of TNF-α and the release of cortisol (Ostrowski et al., 1999; Starkie et al., 2003; Steensberg et al., 2003). In addition, the increased release of cortisol stimulates endogenous glucose production from the liver, while IL-6 has also shown to increase basal and insulin-stimulated glucose uptake in skeletal muscle via stimulation of the 5' adenosine monophosphate-activated protein kinase (AMPK) pathway and associated increase in GLUT4 translocation (Carey et al., 2006; Pedersen & Febbraio, 2008). Overall, the acute inflammatory response to exercise involves inconclusive findings on the potential mechanisms (i.e. regulating the acute release of anti-inflammatory
markers, glycogen depletion and/or immunological strain). Accordingly, these potential mechanisms can be manipulated based on exercise modality, intensity and/or duration. Moreover, research directly comparing the acute inflammatory response between intermittent and continuous exercise in a sedentary cohort, with the aim of increasing the anti-inflammatory milieu is lacking. The respective studies that exist describe the acute mechanistic changes involved across differing exercise protocols, with the majority of literature specific to trained, young men, with minimal literature specific to inactive men. Moreover, for the purpose of stimulating an anti-inflammatory environment and associated glucose regulatory mechanisms additional research is required across mode-specific intermittent exercise within inactive, middle-aged populations.

2.5.2: Chronic inflammatory adaptations to exercise

The acute anti-inflammatory response to exercise shows variable results, which seems to be related to the duration and intensity of the exercise bout and the associated changes in muscle glycogen content (Febbraio et al., 2003; Keller et al., 2001). Furthermore, resting concentrations of IL-6 and CRP have shown an inverse association with muscle mass (Donges et al., 2010; Visser et al., 2002). Although, there is variation in the inflammatory responses to exercise training, which may be a consequence of muscle mass recruitment during each exercise bout and the ensuing muscular hypertrophy adaptation, often which tend to be mode-specific (Coffey & Hawley, 2007; Coffey, Pilegaard, Garnham, O'brien, & Hawley, 2009). However, it should be noted that these body composition adaptations are not consistent, with further variation in inflammatory adaptations to aerobic exercise also stemming from methodological differences, such as the intensity and duration of the training program.
Potentially as a result of widespread clinical use, CRP is the most consistent and published inflammatory marker for exercise-induced training adaptations, compared to other cytokines such as IL-6, TNF-\(\alpha\), IL-1\(\beta\), leptin and adiponectin. Physically active individuals show lower plasma concentrations of CRP and IL-6 when compared to age and gender matched inactive groups (Reuben et al., 2003). Furthermore, within a sedentary population group, a combined decrease in weight and/or fat-mass with increased levels physical fitness may stimulate a greater inflammatory response when compared either factor occurring in isolation (Reuben, Judd-Hamilton, Harris, & Seeman, 2003; You et al., 2013). Recent review papers have collated the effects of exercise training on chronic inflammation across different cohorts and exercise modes (Nimmo, Leggate, Viana, & King, 2013; You et al., 2013). However, the main modes of exercise prescribed have been continuous aerobic conditions (i.e. running and cycle ergometry) and/or strength training, with little evidence incorporating intermittent training.

Pro-inflammatory cytokines are secreted from adipose tissue, with an increase in fat-mass associated with an increase in pro-inflammatory cytokines i.e. CRP and IL-6 (Balducci et al., 2010; Fried et al., 1998; Petersen & Pedersen, 2005). Systemic expression of IL-6 contributes to the hepatic production of CRP, both of which have shown a positive correlation with fat-mass and an inverse association with skeletal muscle mass and aerobic capacity (Donges et al., 2010; Fried et al., 1998; Petersen & Pedersen, 2005; Visser et al., 2002; You et al., 2013). Recent evidence indicates mixed findings in the effectiveness of different modes of exercise to reduce pro-inflammatory markers (Donges et al., 2010; Kohut et al., 2006; Nicklas et al., 2005; Okita et al., 2004). For example, Kohut et al. (2006) reported that a 10 month aerobic training intervention reduced CRP and IL-6 by 33.3% and 37.3% (\(p<0.05\)), respectively, while no significant changes were evident in a combined resistance and flexibility training
intervention (Kohut et al., 2006). This response was further supported by Okita et al. (2004) who found that a two-month aerobic exercise program significantly reduced CRP from 0.63 to 0.41 mg·L\(^{-1}\) (P<0.001). Additionally, this particular study also concluded that the change in CRP was not proportionally associated with the extent of weight reduction (Okita et al., 2004), although there was no reporting of changes in muscle mass and fat-mass in associated with the CRP response.

Furthermore, after 12-weeks of moderate intensity aerobic exercise, Christiansen, Paulsen, Bruun, Pedersen, and Richelsen (2010) did not observe changes in circulating inflammatory proteins IL-6 and adiponectin despite the reported weight loss (3.5 kg). Interestingly, this study followed participants 2 weeks into the detraining period and documented a reversal of circulating IL-6 concentration to pre-training values, pointing to a direct influence of training (Christiansen et al., 2010). Conversely, Donges et al. (2010) reported that an inactive non-diseased population showed a significant reduction in CRP (-32.7 ±27.2%; p=0.05) in response to 12-weeks of resistance training, while aerobic training indicated no significant effect for a reduction (-16.0 ±39.7%; p=0.06), with no change in IL-6. Interestingly, the resistance group was the only group to show an increase in muscle mass, despite no change in fat-mass or aerobic capacity. Accordingly, the equivocal results reported on changes in CRP and/or IL-6 following aerobic training studies may be from a distinct lack in muscle mass accumulation. An explanation of such findings may relate to the preservation of muscle mass through exercise training up-regulating glucose regulatory mechanisms in skeletal muscle (i.e. GLUT4 translocation), which further influence the pro- and anti-inflammatory state to ameliorate metabolic dysfunction (Gleeson et al., 2011; Petersen & Pedersen, 2005). Further, the acute anti-inflammatory response to each exercise bout is driven by muscle mass, thus, an increase in muscle mass may drive a high anti-inflammatory effect of exercise (Pedersen,
2011b). Collectively, the use of training modes that focus on one fitness component (i.e. resistance exercise or continuous aerobic exercise) does not provide the entirety of adaptations known to influence the inflammatory state (i.e. fat-mass, muscle mass and aerobic capacity). Accordingly, selecting training modes that incorporate multiple fitness components may be required for a more substantial and consistent inflammatory adaptations in populations at risk of developing chronic systemic inflammation.

The first two cytokines produced in the pro-inflammatory cytokine cascade are TNF-α and IL-1β, and in addition to IL-6, stimulate the hepatic production of CRP (Petersen & Pedersen, 2005). Despite this inflammatory contribution of TNF-α and IL-1β, the majority of aerobic training studies only report on CRP and/or IL-6. Previous research reporting the response of TNF-α and IL-1β to aerobic exercise training suggest equivocal findings in either healthy or diabetic participants (Balducci et al., 2010; Kadoglou et al., 2007). Specifically, 12 months of continuous aerobic training (70-80% VO2max) reduced CRP (28%), IL-6 (41%), leptin (27%) and increased adiponectin (36%), with no improvements in IL-1β, TNF-α and IL-10. This occurred alongside a reduction in body mass and WC, but without changes in fat- or muscle mass (Balducci et al., 2010). In addition, 6 months of aerobic training (50-75% VO2peak) in diabetic men and women showed no significant changes in TNF-α or adiponectin, including no change in fat-mass, but a decrease in CRP and increase in IL-10 (Kadoglou et al., 2007). Thompson et al. (2010) examined higher intensity aerobic exercise (50-70% VO2max) over 6 months and concluded that a reduction in circulating IL-6 had no influence on resting CRP concentrations. Furthermore, 12-weeks of aerobic training at 75-80% HRmax improved aerobic capacity and fat-mass alongside increases in TNF-α and IL-6, with no change in CRP (Donges et al., 2013). Firstly, the results from these studies show limited data on the IL-1β response to aerobic training. Secondly, the equivocal responses of inflammatory markers to
aerobic training may be a consequence of the different training loads and durations ranging from 12 weeks to 12 months at intensities ranging from 50 to 80% VO$_{2\text{max}}$. Regardless, there seems to be a consistent reduction in the overall pro-inflammatory state and an increased anti-inflammatory environment, as evident through increased IL-10 and adiponectin.

An imbalance of pro- and anti-inflammatory cytokines secreted from adipose tissue contributes to metabolic dysfunction (Arita et al., 1999; Ouchi et al., 2011). Adiponectin is another hormone secreted by adipocytes which stimulates an increase in the anti-inflammatory cytokines IL-10 and IL-1ra in monocytes and macrophages, while inhibiting systemic levels of IL-6 and TNF-\(\alpha\) (Bouassida et al., 2010; Ouchi et al., 2011). The expression of adiponectin protects against metabolic and cardiovascular disorders and is decreased in plasma and adipose tissue in obese, compared to lean individuals (Carson et al., 2012; Ouchi et al., 2011). Furthermore, previous reports suggest a strong positive correlation between the change in IL-1ra and change in muscle mass (Meier et al., 2002). However, in response to aerobic and resistance exercise training there was no change in IL-1ra despite an increase in muscle mass within a 12-week resistance training group (Donges et al., 2013). Despite the anti-inflammatory and antagonistic qualities to the pro-inflammatory marker IL-1\(\beta\), there is limited evidence for the effects of 12 weeks aerobic training on IL-1ra (Donges et al., 2013). Moreover, previous exercise training studies report inconsistent results regarding adiponectin and IL-10 (Balducci et al., 2010; Bouassida et al., 2010; Kadoglou et al., 2007). These studies document an increase in IL-10, which was correlated to a reduction in fat-mass, and no change in adiponectin in response to 6 and 12 months of aerobic exercise training, respectively (Balducci et al., 2010; Kadoglou et al., 2007). Although exercise training alone has been reported to increase adiponectin and IL-10 concentrations, nutritional and diet-based interventions in combination with exercise have been shown to increase these markers more
robustly (Bouassida et al., 2010). Taken collectively, limited evidence is available regarding the anti-inflammatory adaptations to aerobic training. Given adiponectin, IL-1ra and IL-10 have a strong influence on the pro-inflammatory and glucose regulatory state, an examination of these markers in response to aerobic exercise training, in the contest of changes in pro-inflammatory markers and body composition is required. Given the proposed association of these inflammatory cytokines with the development of T2DM and CVD, further research is warranted on training studies that may assist in reducing an over expression of pro-inflammatory cytokines and increasing the expression of anti-inflammatory cytokines within the circulation.

Of recent interest high intensity intermittent training (HIIT) has been implemented within clinical and inactive populations with a focus on improving body composition, whilst also stimulating metabolic and cardiovascular adaptations (Bartlett et al., 2011; Boutcher, 2010). Accordingly, these improvements in body composition and metabolic variables in blood and skeletal muscle provide a rationale and subsequent hypothesis that interval-based training methods may influence the resting concentration of pro- and anti-inflammatory markers. Furthermore, 6 months of high-intensity interval training in patients following successful percutaneous coronary intervention stimulated a decrease in IL-6 and IL-8 and an increase in IL-10, with no change in CRP (Munk et al., 2011). Although this study was conducted in a clinical population, the findings show the potential benefit of interval training for reducing the pro-inflammatory state (IL-6) and increasing the anti-inflammatory state (IL-10) (Munk et al., 2011). Conversely, young active men and women completed 2 weeks of interval (6 x 30 s all out maximal sprint efforts interspersed with 4 min recovery) or continuous (90-120 min cycle ergometry at 65% VO₂max) training 3 d wk⁻¹ (Hovanloo, Arefirad, & Ahmadizad, 2013). Results showed that there was no significant changes within or between conditions
(p>0.05) in inflammatory markers IL-6, CRP and IL-10 (Hovanloo et al., 2013). Although, these results may be a consequence to the short duration of the training intervention and suggest that longer duration of intermittent or continuous training may be required to induce and anti- and pro-inflammatory adaptation.

Furthermore, there are no studies examining the effects of interval-training methods with inactive, disease-free cohorts, particularly with a focus on the chronic systemic inflammatory state. Thus, further research is required to examine the efficiency of different training methodologies with inactive populations. Furthermore, there is conflicting and inconsistent findings associated with changes in pro- and anti-inflammatory markers following continuous and intermittent exercise training. Such experimental variation may be explained by the population group studied and their susceptibility to a pro-inflammatory state (i.e. diabetics compared to health inactive groups), the duration and intensity of the training conditions, differing body composition adaptations relating to fat-mass and muscle mass and methodological differences relating to sample collection, storage and analysis. These confounding factors impair our understanding for the role of aerobic exercise in improving the pro- and anti-inflammatory state. Although conventional training methods such as continuous aerobic training are well researched, additional training modes may be required for additional exercise prescription opportunities for those otherwise impartial to traditionally prescribed continuous gym-based methods.
2.6: Acute and chronic exercise-induced responses for glucose regulation

2.6.1: Acute glucose regulatory responses to exercise

Although the resting concentration of IL-6 has been associated with the development of insulin resistance, Carey et al. (2006) have shown that the acute infusion of IL-6 increases glucose disposal without affecting the suppression of endogenous glucose production during a hyperinsulinaemia-clamp in healthy participants. Furthermore, in vivo treatment of IL-6 has been shown to enhance basal and insulin-stimulated glucose disposal and cortisol expression, while in vitro IL-6 might increase GLUT4 translocation to the plasma membrane and AMPK activity (Carey et al., 2006; Kelly et al., 2004; Pedersen & Fischer, 2007; Steensberg et al., 2003). Furthermore, cortisol is a stimulatory hormone that contributes to increased hepatic glucose production (Kindermann et al., 1982), and IL-6 stimulates peripheral glucose metabolism via stimulation of the AMP-kinase pathway and associated increase in GLUT4 translocation (Carey et al., 2006; Helge et al., 2003; Pedersen & Febbraio, 2008). Accordingly, an acute increase in systemic concentrations of IL-6 has been shown to mediate the hepatic glucose output and peripheral glucose metabolism necessary to maintain blood glucose homeostasis when the uptake of glucose by skeletal muscle is required during prolonged exercise bouts (Gleeson, 2000; Nybo et al., 2002; Pedersen, et al., 2001).

These potential effects of IL-6 for stimulating energy metabolism, Keller et al. (2001) sought to determine whether energy availability influenced the regulation of IL-6 expression in skeletal muscle and in plasma. Results demonstrated that low glycogen amplified plasma IL-6 concentration and mRNA expression (Keller et al., 2001). Furthermore, irrespective of exercise mode (i.e. cycle ergometry and running) exercise elicited in a 21-fold increase in IL-6 mRNA expression (Starkie, Arkinstall, Koukoulas, Hawley, & Febbraio, 2001). In contrast,
while the mode of exercise did not affect the exercise-induced increase in plasma IL-6, carbohydrate ingestion and the consequential increase in plasma glucose concentration (rest ~4.6 mmol·L⁻¹ to ~ 5.4 mmol·L⁻¹ after glucose ingestion) blunted this response (p<0.01) (Starkie et al., 2001). Collectively, these findings show that energy availability affects the acute IL-6 response, regardless of exercise mode. Further, the resultant IL-6 expression and plasma concentration in response to acute exercise may provide positive alterations to metabolic processes via increased cellular glucose uptake (Chan, Carey, Watt, & Febbraio, 2004b; Keller et al., 2001; Pedersen, 2006; Pedersen & Febbraio, 2008; Starkie et al., 2001; Walsh et al., 2011).

Independent to the acute inflammatory response, the utilisation of intramuscular glycogen stores and glucose delivered from the plasma becomes an increasingly important energy substrate to the working muscle with an increase in exercise intensity – for further information, the process of glucose regulation and metabolism during and after exercise is extensively reviewed in Jensen and Richter (2011). The rate of glucose disposal and the immediate post-exercise response in insulin sensitivity can be related to two distinct mechanisms, including the type of muscle contraction (i.e. concentric and eccentric) and/or the energy requirements throughout the exercise (i.e. continuous and intermittent). For example, continuous aerobic exercise such as stationary cycle ergometry is indicative of lower-body concentric muscular contractions, when compared to intermittent running that encourages high-intensity sprints of an eccentric nature. Accordingly, the acute regulatory response to exercise may be dictated by these mode specific characteristics. However, no studies have directly compared the glucose regulatory differences between these exercise modes of concentric continuous aerobic cycle ergometry compared to intermittent running, inclusive of eccentric loading.
Firstly, eccentric contractions and associated muscle damage has been shown to decrease insulin sensitivity immediately post-exercise, compared to concentric exercise which increases insulin sensitivity (Asp, Daugaard, Kristiansen, Kiens, & Richter, 1996). Eccentric exercise represents dynamic exercise involving forces lengthening the contracting muscle (Asp et al., 1996). As an example of the interaction of contractile type with glucose regulation, a previous study in humans reports that unaccustomed eccentric exercise lead to whole-body insulin resistance (Kirwan et al., 1992). Moreover, eccentric exercise transiently decreases the skeletal muscle GLUT4 protein content and impairs post-exercise glycogen resynthesis (Asp, Daugaard, & Richter, 1995; Costill et al., 1990; Zehnder, Muelli, Buchli, Kuehne, & Boutellier, 2004). Specifically, Asp et al. (1996) studied the effects of one-legged eccentric exercise on insulin action in muscle. Results demonstrated that whole-body and muscle glucose uptake was impaired during maximum insulin stimulation after eccentric exercise, with no effect at sub-maximal insulin concentrations. These findings suggest that muscle subjected to unaccustomed eccentric exercise becomes resistant to maximal insulin action (Asp et al., 1996).

Conversely, a single bout of concentric exercise (shortening of the contractile apparatus) has been shown to enhance insulin action in muscle (Bogardus et al., 1983; Richter, Mikines, Galbo, & Kiens, 1989). Indeed, after one-legged glycogen depleting concentric knee extension exercise, glycogen resynthesis was markedly increased compared to the non-exercised leg (Bogardus et al., 1983; Richter et al., 1989). Following this, Richter et al. (1989) showed in a human model that local concentric contractions increased response to a sub-maximal insulin concentration and overall insulin sensitivity in the working skeletal muscle. Further, these results also demonstrated the importance of increased insulin sensitivity for post-exercise recovery through glycogen resynthesis (Richter et al., 1989). In
summary, it seems that the specific type of contractile demands of exercise (i.e. eccentric and concentric contractions) differentially stimulate insulin sensitivity, which in turn alters glucose uptake and the rate of glycogen resynthesis during the post-exercise recovery period. To date, the majority of literature has focused on eccentric and concentric contractions using one legged exercise models. While this provides physiological relevance into the effects of different muscle contractions it does not provide evidence of practicality for the purpose of exercise prescription.

In addition to eccentric versus concentric contractions, the notion of exercise type affecting regulation of glucose disposal can also be influenced by intermittent and continuous exercise (i.e. cycle ergometry). In particular, intermittent exercise of transient but high-intensity bouts are characterised by increased rates of glycolysis and associated carbohydrate metabolism and glycogen depletion (Christmass, Dawson, Passeretto, & Arthur, 1999). Brestoff et al. (2009) showed that an acute bout (45 min at 75% VO$_{2peak}$) of continuous cycle ergometry increased insulin sensitivity by 70-100% (p<0.05). Comparatively, high-intensity interval cycling (5 supra-maximal sprints at ~125% VO$_{2peak}$, separated by 4-5 min recovery) showed no significant change in insulin sensitivity at 40-80% relative to baseline (p>0.05) (Brestoff et al., 2009). Although speculative, these results could in part be explained by suppressed insulin sensitivity during the post-exercise period that resulted from the eccentric loading during the high-intensity intermittent sprints (Kirwan et al., 1992). In addition to Brestoff et al. (2009),

Campbell et al. (2014) compared intermittent running (simulated games activity, intermittent sprints interspersed by continuous running) with continuous running in those with diagnosed type 1 diabetes mellitus (T1DM). The different glucose regulatory capability in those with
T2DM makes it difficult to extrapolate glucose responses to inactive, normo-glycemic populations. However, it is acknowledged that this study provides the possibility for assessing the glucose regulatory benefits of acute intermittent exercise in a sports-specific manner (Campbell et al., 2014). Regardless, only one study directly compares insulin sensitivity between intermittent and continuous eccentric and concentric cycle ergometry within, disease-free, inactive men (Brestoff et al., 2009). Although this is a common mode for exercise prescription that has been extensively research (Garber et al., 2011), different modes of intermittent exercise (i.e. running) and the systemic glucose regulatory response requires further investigation.

2.6.2: Systemic and skeletal muscle glucose regulation and mitochondrial biogenesis relating to exercise training

Skeletal muscle is a crucial tissue for maintaining blood glucose control and energy balance (McPherron, Guo, Bond, & Gavrilova, 2013). In addition to eccentric and concentric contractions, differences between conditions involving intermittent and continuous exercise have shown to stimulate different energy requirements and thus different glucose regulatory responses to an exercise bout (Christmass et al., 1999). Krstrup et al., (2004) assessed changes in single-fibre metabolites during sub-maximal, dynamic exercise at two intensities to evaluate fibre recruitment. Glycogen depletion patterns confirmed that both Type I and II muscle fibres were active during intense exercise, whereas Type I fibers were recruited at moderate intensity (Krustrup et al., 2004). These results demonstrate that fluctuation in exercise intensity reflect changes in fibre type recruitment and associated changes in the metabolic contribution to the exercise bout (Krustrup et al., 2004; Krstrup et al., 2009; Krustrup et al., 2010d). Accordingly, the different energy and fuel source requirements between intermittent and continuous aerobic exercise creates a further divide in the glucose
regulatory adaptations between modes. Factors that affect fuel selection and metabolic responses in continuous and intermittent exercise include muscle fibre recruitment, prior training, diet, and the intensity, duration and mode of exercise (Krustrup et al., 2004; Krustrup et al., 2009; Krustrup et al., 2010d; Lindholm, 2007). These factors collectively influence the extent to which chronic adaptations occur and potentially influence systemic and skeletal muscle glucose regulation and mitochondrial biogenesis (Lindholm, 2007).

Similar to the acute glucose regulatory response, there are only a few studies having directly compared systemic glucose regulatory adaptations between intermittent and continuous exercise across trained and untrained cohorts. Earnest et al. (2012) compared 3 months of treadmill running (3-4 times per week, 12 kcal/kg/wk) using continuous (50-70% VO2max) or interval (1:1, work to rest ratio of intervals lasting 2 min, 90-95% VO2max) methods in sedentary, obese men (30-60 y). Both continuous and interval groups similarly improved fasting glucose, OGTT (glucose and insulin) and VO2max, while the interval training had a greater effect on HOMA-IR at 24 h and 72 h post-exercise, when compared to no changes noted in the continuous group (p<0.05). These observations are important as they represent the accumulative effect on glucose uptake and insulin signaling (Hawley & Lessard, 2008). Additionally, 6 weeks of sprint interval (4-6 maximal sprint efforts, 3 times per week) compared to continuous (40-60 min ~65% VO2peak, 5 d/wk-1) cycle ergometry in young sedentary men (21 y) showed comparable improvements between conditions in insulin sensitivity (Matsuda ISI), glucose AUC and insulin AUC, including a comparable increase in VO2peak and no change in BMI (Cocks et al., 2012). In comparison, two weeks of high-intensity training (4-6 x 30 s maximal sprint efforts, 6 d/wk-1) in sedentary young men (21 y) showed a reduced glucose AUC (12%), and insulin AUC (37%) although there was no control or alternative training comparison to the HIIT condition (Babraj et al., 2009). Both
Earnest et al. (2012) and Cocks et al. (2012) compared HIIT to continuous training using different modes of running and cycle ergometry, respectively. The clear difference in training load and duration makes it difficult to compare results between modes and studies; however, it does show that incorporating high-intensity efforts into training induces a beneficial glucose regulatory adaptation at a systemic level within a sedentary population.

The systemic glucose regulatory adaptation to both HIIT and continuous endurance training has shown beneficial results within sedentary populations. Evidence by Nassis et al. (2005) indicated that 12 weeks of aerobic training (a range of continuous, intermittent and sports-specific exercises) improved insulin sensitivity in 19 overweight and obese girls. This study also demonstrated training-induced adaptations in aerobic capacity (+18.8%, p<0.05), and reduced the insulin response (i.e. decreased insulin AUC) (Nassis et al., 2005). Other improvements in glycemic control including improved HbA1c, fasting glucose, insulin sensitivity and glucose AUC in response to an OGTT have also been reported with HIIT (Marwick et al., 2009). Despite these glycemic changes, the proposed mechanisms for improved glucose regulation remain unknown. Specifically, no studies have examined the changes in mitochondrial or skeletal muscle proteins potentially, which may be partially responsible for the changes in glucose control.

GLUT4 and Akt are identified as a key glucose transporter responsible for insulin and contraction stimulated glucose transport into skeletal muscle (Wu et al., 2011). Since the over expression of GLUT4 and Akt protein content in skeletal muscle is associated with enhanced glucose disposal and insulin action, there has been considerable interest in therapeutic strategies, such as exercise training, to increase GLUT4 expression and the associated systemic glucose disposal and control (Egan & Zierath, 2013; Gonzalez & Mcgraw, 2006;
Pessin, Thurmond, Elmendorf, Coker, & Okada, 1999; Richter & Hargreaves, 2013). For example, Nordby et al. (2012) assessed the systemic glucose regulatory response and GLUT4 adaptations in total protein content in sedentary, overweight men completing 12 weeks (3-4 sessions per week) of endurance training (i.e. cycle ergometry, running, rowing at ~65% HR-reserve) with HIIT (5-6 bouts of 3-4 min at 85% HR-reserve). The main finding of this study was that endurance training has beneficial dual actions on body composition (increased muscle mass, decreased fat-mass), increased aerobic capacity and increased peripheral insulin sensitivity of glucose metabolism in skeletal muscle – potentially through increased GLUT4 protein content (insulin stimulated glucose clearance) (Nordby et al., 2012).

It is well established that both HIIT and continuous exercise training improves insulin sensitivity in participants with and without T2DM. The study by Nordby et al. (2012) compares these systemic adaptations in association with GLUT4; however, Akt was not measured and has not been measured in association with this combination of HIIT and aerobic training adaptations. Improvements in insulin sensitivity and glucose uptake have been shown to be facilitated by increased GLUT4 and Akt content (Gonzalez & Mcgraw, 2006; Goodyear & Kahn, 1998). An important function of Akt is to mediate the metabolic actions of insulin to stimulate cellular glucose transport (Frøsig et al., 2007; Whiteman et al., 2002). Given that GLUT4 is highly abundant in skeletal muscle and is associated with enhanced glucose disposal and insulin action, there has been extensive interest in therapeutic strategies to increase Akt and GLUT4 expression in cohorts at risk of developing metabolic disorders (Froesig et al., 2007; Whiteman et al., 2002). Therefore it seems pertinent to assess changes associated with more specific intermittent training in relation to changes in GLUT4 and Akt content and ensuing systemic glucose regulatory adaptations (Christ-Roberts et al., 2004). Regardless, these collective results demonstrate a concomitant change in systemic
adaptations within skeletal muscle glucose regulatory adaptations (i.e. GLUT4 and Akt protein content) in a sedentary cohort. Furthermore, these studies demonstrate that endurance exercise *per se* increases various metabolic health parameters and thus endurance training should also be included in an intervention aimed at improving metabolic health in overweight, inactive cohorts.

On the topic of endurance training, for many years it was thought that the increase in the capacity for endurance exercise was exclusively a result of cardiovascular adaptations as characterised by an increased capacity to deliver oxygen to the working skeletal muscle (Holmgren & Strom, 1959). Whilst cardiovascular adaptations are still a major component of endurance adaptations, Holloszy (1967) recognised that an increase in skeletal muscle mitochondria content and activity were adaptations that can partially account for an increase in aerobic capacity. PGC-1α is a critical regulatory gene for mitochondrial biogenesis in skeletal muscle and promotes biogenesis in response to exercise to maintain a balance between energy requirements and energy supply (Wu et al., 1999). The relationship between glucose regulation and mitochondrial biogenesis becomes important when assessing skeletal muscle mechanism that may be responsible for clinical glucose regulatory and aerobic capacity outcomes (Choi et al., 2008).

Several studies have compared skeletal muscle adaptations relating to glucose regulation and mitochondrial biogenesis between intermittent and continuous exercise. Specifically, 8 type 2 diabetics completed 6 HIIT sessions (10 x 60 s cycle ergometry bouts ~90% HR_{max}, interspersed with 60 s rests) over a 2 week period. Results demonstrated improved glucose regulation over a 24 h period in response to a standardised breakfast, lunch and dinner (Little et al., 2011). In addition, skeletal muscle adaptations included higher citrate synthase activity
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(~20%) and an increase in the content of COX II, COX III, COX IV and GLUT4 proteins (Little et al., 2011). Similarly, in healthy, sedentary, men (n=4) and women (n=3) performing a comparable HIIT protocol, increased muscle oxidative capacity was observed, as reflected by the protein content of citrate synthase and cytochrome c oxidase subunit IV (35%). Furthermore, after training there was an increase in protein content of PGC-1α (56%) and GLUT4 (260%), coinciding with improved systemic insulin sensitivity (HOMA-IR) (35%), (Hood, Little, Tarnopolsky, Myslik, & Gibala, 2011). Collectively, these results indicates that a low-volume but high-intensity (i.e. HIIT) training program in type 2 diabetics improves glucose control and induces adaptations in skeletal muscle that are linked to improved metabolic health (Little et al., 2011). However, as stated previously, these HIIT specific studies do not measure the protein content of Akt, which is an additional protein responsible for systemic glucose regulation.

Interestingly, these chronic skeletal muscle adaptations are not consistent between studies, with some studies reporting no change in protein content (Donges et al., 2013; Hood et al., 2011; Skleryk et al., 2013). Specifically, the only study that directly compares 2 weeks of HIIT and continuous training in sedentary (overweight or obese), middle-aged men reports no changes within or between groups after training in body composition (BMI, WC, fat-mass, muscle mass), VO$_{2}$peak, glucose, insulin and HOMA–IR (Skleryk et al., 2013). Furthermore, there were also no changes in skeletal muscle markers of glucose metabolism and mitochondrial function (COX II, COX IV, GLUT4 and SIRT1), which not unexpectedly remained unaltered after only 2 weeks of exercise training (Skleryk et al., 2013). This is further supported by Cochran et al. (2014) who reports that 6 weeks (3 days per week, total of 18 sessions) of interval (4 x 30 s maximal sprint efforts, interspersed with 4 min recovery ~1.0 kJ per kg of body mass) or continuous (60 min ~65% VO$_{2}$peak at ~75-100 W) training
stimulated no change in GLUT4 protein content and a moderate increase in COX IV (20%) alongside increased VO_{2peak}. A limitation in this study is that there were no measures of PGC-1α or systemic glucose regulatory variables. Collectively, these studies demonstrate a divergent and mixed understanding of the skeletal muscle adaptations. A notable reason for this could include differences in training duration and the lack of glucose regulatory and mitochondrial adaptations (systemic and skeletal muscle) in response to 2 weeks of training (Skleryk et al., 2013). Further, there is a lack of literature linking systemic clinical outcomes to skeletal muscle adaptations in relation to glucose regulation and mitochondrial biogenesis in response to different modes of exercise (i.e. interval and continuous training). Furthermore, the inconsistent results between the literature and the lack of comparisons between intermittent and continuous protocols demonstrates a need for future research that explores a more practical approach to intermittent exercise as opposed to these previous studies that solely focus on cycle ergometry.

When comparing skeletal muscle adaptations between intermittent and continuous training, all studies have used cycle ergometry in active and inactive, young men and women (Burgomaster et al., 2008; Burgomaster, Hughes, Heigenhauser, Bradwell, & Gibala, 2005; Gibala, 2007; Hood et al., 2011). For example, Burgomaster et al. (2008) compared the responses of young untrained men (n=5 per group) and women (n=5 per group) completed 6 weeks (3 days per week) of high-intensity interval (4-6, 30 s maximal sprint efforts with 4.5 min recovery, 3 days per week) or continuous (40-60 min, ~65% VO_{2peak} 5 days per week) cycle ergometry training. Results showed the maximal activity of citrate synthase and 3-hydroxyacyl CoA dehydrogenase (β-HAD) and the total protein content of pyruvate dehydrogenase E1α (PDH) and PGC-1α were similarly increased after training in both conditions (Burgomaster et al., 2008). Furthermore, Gibala et al. (2006) examined the
response of 16 active men in response to 6 training sessions over 14 d of HIIT (4-6, 30 s maximal sprint efforts with 4 min recovery, 3 days per week) or continuous (90-120 min, ~65% VO$_{2peak}$ 5 days per week) cycle ergometry training. Results reflected increased maximal activity of COX and COX subunits II and IV protein content including an increase in glycogen content. Taken as a whole, these data suggests increased mitochondrial activity and/or content in response to HIIT was comparable to traditional continuous exercise in active and untrained young men. However, the application of these adaptations within inactive, overweight, middle-aged men remains vastly under researched.

Endurance exercise training induces numerous morphological and metabolic adaptations in skeletal muscle, including mitochondrial biogenesis and glucose metabolism (Holloszy, 1967; Holloszy & Booth, 1976). These adaptations to exercise training have significant clinical relevance for improved metabolic health (Hawley, 2004; Warburton, Nicol, & Bredin, 2006a). As discussed previously a range of proteins responsible for mitochondrial biogenesis have been assessed in response to intermittent and continuous training. However, additional proteins responsible for the activity of PGC-1α and potentially mitochondrial biogenesis have been recently identified, including SIRT1 and p53 (Kim, Kho, Kang, & Um, 2007; Manoli et al., 2007; Rodgers et al., 2008; Saleem, Adhihetty, & Hood, 2009; Saleem & Hood, 2013). Moreover, there is limited literature available on the SIRT1 response to exercise in humans, with only two training studies documented on HIIT and none on continuous exercise modes. Specifically, following 6 weeks of interval training there was a 16% increase in PGC-1α protein content, although, SIRT1 increased in activity (31%) rather than content (-20%) (Gurd, Perry, Heigenhauser, Spriet, & Bonen, 2010). In contrast, 2 weeks of HIIT training increased SIRT1 (56%) content, with no changes in PGC-1α content. Furthermore, there was an increase in PGC-1α nuclear abundance (~25%) and increased total
protein content of citrate synthase, COX subunits II and IV, Tfam and GLUT4 (Little, Safdar, Wilkin, Tarnopolsky, & Gibala, 2010). More recently, using rat models have demonstrated that p53 is a potential acute regulator of mitochondrial content and function (Bartlett et al., 2012), although there are no published studies involving exercise training in humans. As such, the measurement of p53 may provide further information regarding the mitochondrial adaptations to intermittent and continuous exercise.

In summary, additional mechanisms relating to impaired glucose regulation and insulin signalling have involved the functioning and content of key regulatory proteins responsible for mitochondrial biogenesis (Finck & Kelly, 2006). Accordingly, it may be a combination of these mechanisms that contribute to improving blood glucose metabolism through intermittent or continuous exercise. Mitochondrial function relates to the protein content, specific volume and rate of aerobic oxidation capable by the skeletal musculature (Hoppeler & Fluck, 2003). As such, further research is required to investigate the chronic muscle based mechanisms potentially responsible for increased mitochondrial functioning as related to clinical markers of glucose regulation. In turn, these areas may provide a greater understanding of a decreased susceptibility to chronic disease development through improved systemic glucose control and disposal (Wright et al., 2006).

2.7: Exercise interventions within Indigenous populations

The previous sections have almost exclusively dealt with research on cohorts specific to Caucasian or of an undisclosed ancestry background. Accordingly, there is almost a complete absence of research into exercise, inflammation and glucose regulation in Indigenous Australian populations. However, given the exacerbation in disease prevalence rates and
poorer health quality in the Indigenous communities, some discussion of the role of exercise is pertinent. Physical activity interventions such as walking, running or reverting back to a notion of ‘hunter-gather’ lifestyles amongst Indigenous populations around the world have been met with variable outcomes. Moreover, exercise interventions that have applied single exercise modes have generally been met with limited success across Hispanic, Asian, and Native American cohort (Venkat Narayan et al., 1998; Wing et al., 2004). However, community- or sports-based exercise programs have led to consistent health improvements across the literature involving different Indigenous populations, often manifesting in improved body composition and glucose regulatory variables (Biddle et al., 2011; Canuto, Cargo, Li, Esterman, & Mcdermott, 2012; Foulds, Bredin, & Warburton, 2011; Heath, Leonard, Wilson, Kendrick, & Powell, 1987; Heffernan et al., 1999; Kochevar, Smith, & Bernard, 2001; Rowley et al., 2000a). Despite the relatively limited literature, these results suggest a community-based intervention is likely to be more successful among Indigenous populations than isolated or individual based training programs. Given the high rates of obesity, diabetes, CVD and other chronic conditions amongst these Indigenous populations, it has been suggested community-based physical activity interventions should be implemented to combat the poor health status these populations experience (Anand et al., 2001; Boulé et al., 2005; Brown, 2012; Thomson et al., 2011; Trewin & Madden, 2005; Wang et al., 2007).

For the purpose of this thesis, the focus will be specific to Indigenous Australians with the prospect of investigating community interventions to increase levels of physical activity for improved health indices, and to contribute to preventing the development of metabolic and cardiovascular abnormalities. Early studies reporting on glucose, insulin and body composition within the Indigenous Australian communities (prior to European colonisation) revealed fasting glucose \((3.8 \pm 0.4 \text{ mmolL}^{-1})\) and cholesterol \((3.9 \pm 0.2 \text{ mmolL}^{-1})\)
concentrations were low relative to urbanised Indigenous and Caucasian Australians (O'dea, Spargo, & Nestel, 1982). However, fasting insulin concentrations (13 ±4 mUL⁻¹) were similar to westernised Indigenous Australian men and higher than Caucasian men, both of which had higher levels of BMI (~21 kg m⁻²) (O'dea et al., 1982). These data suggest that in view of their low BMI (~17 kg m⁻²) and low fasting glucose, their insulin levels were inappropriately elevated. Additionally, the Indigenous Australians also had higher than normal fasting triglycerides concentrations based on relative extreme leanness in observed body morphology (O'dea et al., 1982). Collectively, these results also show that westernised Indigenous Australians are in a poorer metabolic status when compared their status prior to European colonisation. This evidence of insulin resistance despite extreme leanness and physical activity suggests an increased susceptibility to obesity and T2DM.

Following these descriptive results, O'dea (1984) attempted to temporarily reverse European influences within a group of urbanised Indigenous Australians by reverting participants back to hunter-gather lifestyle for 7-weeks (O'dea, 1984). Results in the non-diabetic Indigenous participants, showed improved glucose tolerance to a glucose load, albeit without changes in fasting glucose and insulin concentrations. Furthermore, glucose AUC decreased in these participants with no change in insulin AUC (O'dea, 1984). These authors concluded that at least three factors known to improve insulin sensitivity (weight loss, low-fat diet and increased physical activity) would have caused the metabolic changes observed, even without changes in insulin concentration (O'dea, 1984). Although, increased levels of physical activity did not normalise insulin concentrations, there was an improvement in metabolic control to a glucose load. Accordingly, these results indicated that increasing levels of physical activity within these communities may allow for improved metabolic control and a
reduction in risk factors associated with the development of metabolic and cardiovascular abnormalities.

As further evidence for the role of exercise in Indigenous communities, Rowley et al. (2000a) assessed the sustainability and effectiveness of community-directed programs for the primary and secondary prevention of obesity, diabetes and CVD in Indigenous Australians. This study concluded that developmental initiatives facilitating planning, implementation and ownership of interventions by community members and organisations can be a feasible and effective way to achieve sustainable improvements in health behaviours and selected health outcomes among Indigenous people (Rowley et al., 2000a). In addition, Rowley et al. (2000a) incorporated dietary education, in addition to physical activity which was promoted through regular hunting trips, participation in sport (basketball or football, 2-3 sessions per week) and regular walking groups (3-4 times per week, one hour sessions). The outcomes to this study were reductions in BMI, fasting glucose, fasting insulin, 2 h glucose concentration after an OGTT, with repeated measures obtained over a 24 months period (0, 6, 12, 18 and 24 months). The follow-up program within this community was further directed towards sport-based participation, delivered through the employment of sport officers for the running of local tournaments and festivals. These festivals were delivered with a focus on ‘Fitness Fights Diabetes’, with a significant trend over a 4 y period for an increase in those reporting to be physically active and a subsequent decrease in those reporting to be inactive (Rowley et al., 2000a). Collectively, while this study incorporated several approaches for improving health in these Indigenous communities, the incorporation of sport has produced further interest within other Indigenous populations.
Biddle et al. (2011) also implemented team-sports for aerobic capacity and health benefits in Pacific Adults showed positive outcomes. Untrained men and women were offered 45 minutes of SSG (soccer, basketball, touch rugby, and cricket) 3 d wk\(^{-1}\) over a four week period. Results demonstrated an increase in VO\(_{2\text{peak}}\), leg strength and high-density lipoprotein (HDL) when compared to an inactive control group. The authors concluded that SSG may be a promising means for improving health and aerobic capacity and reducing risk of diabetes and CVD in Pacific adults (Biddle et al., 2011). It is difficult to compare the health outcomes between these two studies of different populations; however, both of these studies conclude that community-based exercise interventions through sport (particularly as SSG) may be an appropriate avenue to consider for improved health benefits. These previous studies conducted dietary awareness and education, however this does not provide results on the physiological benefits of exercise \textit{per se}, nor the mechanisms underlying any improvement in the prevention of disease development.

Indigenous Australians experience a high resting inflammatory state, which is suggested to be a potential mechanism for high rates of glucose abnormalities and overall chronic disease development (Alberti et al., 2006; Hodge et al., 2010; Rowley et al., 1997; Wang & Hoy, 2007). In more recent years, Canuto et al. (2012) showed that 12-weeks of grouped gym sessions resulted in a modest reductions in weight, BMI, and blood pressure within inactive Indigenous Australian women. In addition, further improvements in these measures were observed 3-months following the original intervention. It is important to recognise that there were no changes in WC, WHR, and fasting variables of glucose, insulin, calculated HOMA-IR, cholesterol and CRP following this time frame of training. Over the 12 weeks, the attendance rates to the exercise classes were 40\%, which may provide reason for these minimal physiological adaptations reported. This study concluded that structured exercise
Chapter 2: Review of Literature

programs implemented in community settings requires greater attention to understand the barriers for participation within these Indigenous groups (Canuto et al., 2012). Collectively, these interventions report on the health benefits of both grouped gym and sports-specific training sessions, with different adaptations potentially relating to session attendance and ultimately the overall training stimulus. In addition to low adherence rates, a limitation with recent studies is that CRP was the only inflammatory marker measured, including no assessment of glucose regulation to a standard glucose load (i.e. OGTT) (Canuto et al., 2012). It is suggested that the variable and pleiotropic activities of cytokines means it to be even more essential to measure multiple markers within the systemic inflammatory cascade to ensure an overall inflammatory analysis at rest and in response to exercise is obtained. Furthermore, this study is specific to Indigenous Australian women, with no literature available on men. For these reasons it is of importance to extend such research to assess the inflammatory response with reference to glucose regulatory and anthropometric variables within Indigenous Australian men.

The Indigenous Australian population has higher rates of physical inactivity, obesity and chronic disease when compared to their non-Indigenous counterparts. Furthermore, central fat deposition is highly associated with increased systemic inflammatory state (Nakamura et al., 2008). Accordingly, high concentrations of CRP in Indigenous men and women were explained by abdominal obesity (Hodge et al., 2010). A notable finding by Hodge et al. (2010) was that WC or WHR are preferred obesity measures to appropriately reflect cardiometabolic risk in Aboriginal Australians, who although leaner by BMI criteria, displayed a similarly adverse risk profile to Aboriginal Canadians. Accordingly, it is suggested that WC and/or WHR should be routinely included in clinical assessment in these high-risk populations (Maple-Brown et al., 2013). Whilst to date no direct comparison has
been made between Indigenous Australians and Caucasians, these results in addition to the high rates of inflammatory related disease in Indigenous Australians, suggests the notion that resting inflammatory and anthropometric variables may differ to those reported in Caucasian populations. That said, it should also be noted that there are no acute exercise studies specific to an Indigenous Australians population. Assumingly, it is these acute responses to different exercise modes that assist with the development of evidence-based best-practice for exercise prescription to induce appropriate health benefits. However, the majority of research addressing acute mode-specific responses relating to inflammation and glucose regulation comes from cohorts of undisclosed ancestry or Caucasian specific populations (as discussed in previous sections). Given the high rate of disease and lower life expectancy within Indigenous Australians it would be even more pertinent to explore this acute physiological response to exercise to further understand the chronic adaptations to improve health and fitness and limit disease development. Accordingly, for the purpose of this thesis, a focus on inflammation and glucose regulation will be addressed.

2.7.1: A new role for exercise in Indigenous communities

It has been well documented that Indigenous populations in developed ‘post-colonial’ nations (such as Australia, New Zealand, Canada, and the United States) experience disadvantage in a number of areas when compared with their non-Indigenous counterparts (Albert, 2007; Anand et al., 2001; Anand et al., 2000; Katzenellenbogen et al., 2012; Macdonald, Abbott, & Jenkins, 2012; Minges et al., 2011; Naqshbandi et al., 2008; Ring & Brown, 2002; Saad et al., 1991). Despite (or perhaps because of) a range of initiatives to address their disadvantage, there continues to be poor understandings of what ‘works’ and under what conditions (Sweet, 2010). However, there is insufficient engagement of Indigenous people in the research, in particular Indigenous Australians. Accordingly, Carson and Koster (2012) recently proposed
a more rigorous approach to comparative research that is based on principals of inclusive partnership with and participation of Indigenous people. Furthermore, comparative research can not only provide new insights to old problems (Carson & Koster, 2012), it can also, for the purpose of this thesis address the physiological benefit for prescribing exercise within Indigenous Australians and the physiological differences this response may have when compared to a Caucasian population.

Indigenous Australians consider exercise as a means to interact with their family and the larger Aboriginal community (Thompson et al., 2000). These social connections are more important than individual health benefits that solitary exercise may provide (Thompson et al., 2000). If exercise is done solely for the benefit of the individual, it is often considered shameful or as disconnecting individuals from ties within their social world (Thompson et al., 2000). The effectiveness of any intervention to engage and/or change high-risk community behaviour associated with disease development is reported to depend on social importance and relationship to community and social meanings (Thompson et al., 2000). The transference of research findings of the beneficial effect of exercise tend to contain a specific ethnic bias in assumptions relating to availability of such equipment and social importance to engage in health-related exercise training. A common difficulty within the Indigenous Australian population is the lack of facilities to promote the conventional approach to exercise training through gym-specific methods of aerobic (i.e. cycle ergometry or running) and resistance-based exercises. These types of structured exercise training programs provide physiological benefits specific to inflammation, glucose regulation and body composition. However, the limitation with implementing these conventional programs within regional Indigenous Australian populations is that is does not represent the communities’ physical activity patterns, interest and/or understanding of community involvement into one’s health.
As such, traditional notions of gym-based exercise modes as a sustainable and effective intervention to reduce disease risk may be questionable, especially in Indigenous populations.

In particular, Australian football and rugby league is of highest popularity amongst Indigenous Australians (Neesham & Garnham, 2012), and thus creates and opportunity for encouraging community engagement and ownership over an exercise program. However, as yet this sports-specific approach to exercise training in Indigenous Australians does not exist. Thus investigating this method of exercise prescription may provide an under researched populations with high rates of physical inactivity, obesity and chronic disease with an opportunity of evidence-based research for exercise prescription, specific to their cultural and community needs. Not only that, the notion of SSG represents a concurrent inclusion of the virtues of both high-intensity intermittent like exercise over prolonged durations reminiscent of continuous endurance exercise – ideally in a palatable form to ensure sufficient adherence.

2.8: Small-sided games as a method for exercise prescription

Football is a popular team sport that contains positive motivation and social factors that facilitate individual compliance and adherence for maintaining a physically activity lifestyle (Andersen et al., 2010b; Elbe, Strahler, Krstrup, Wikman, & Stelter, 2010; Nielsen et al., 2014; Ottesen, Jeppesen, & Krstrup 2010). The popularity of football codes, regardless of the specific sport, provides a potential vehicle for the awareness and prevention of factors leading to chronic disease, not only for individual health but also the health of society as a whole (Blatter & Dvorak, 2010; Elbe, Strahler, Krstrup, Wikman, & Stelter, 2010; Nielsen et al., 2014; Ottesen, Jeppesen, & Krstrup 2010). Accordingly, the popularity of football is significant to the communities as a whole, not just to the Indigenous populations, who
historically have high social engagement with football codes (Neesham & Garnham, 2012). For these reasons, the use of football as a modality for exercise prescription may be an achievable panacea for low levels of physical activity within both Indigenous and non-Indigenous communities. Rugby league is a football code of high popularity in the region in which research for this thesis was undertaken (regional New South Wales, Australia). Notably, recent studies have shown that regular SSG (futsal) in participants with T2DM improves VO$_{2peak}$, reduces fat-mass, and may positively influence glycemic control (Andersen et al., 2014; de Sousa et al., 2014; Schmidt et al., 2013). However, to date there is minimal research on the physiological health benefits of using rugby as a mode to improve risk-factors associated with the prevention of T2DM and CVD in sedentary cohorts.

Exercise in the form of SSG involves the training of multiple fitness components relating to speed, endurance and strength in the form of intermittent high-intensity sprints interspersed with a high-volume of low-intensity running (Krstrup et al., 2010a; Nybo et al., 2010; Randers et al., 2010b). As shown in Figure 2.3, training across multiple fitness components through SSG has the potential to induce cardiovascular, body composition, skeletal muscle and glucose tolerance for the overall prevention of lifestyle related diseases such as CVD and T2DM (Krstrup et al., 2010a). For these reasons SSG as a potential model for exercise prescription in physically inactivity communities may provide a substantial physiological stimulus that is better than or equal too more traditional exercise modes predominantly focusing on one training component i.e. moderate intensity continuous aerobic exercise applied as running or cycle ergometry.
Figure 2.6
Outcomes and relationships between components of training and the associated adaptation’s for reducing the risk of lifestyle related diseases. Full lines: well know/comprehensive relationships; Dotted lines: Sub-optimal yet positive effects.
2.8.1: Acute responses to small-sided games in untrained populations

There has been increasing interest in the acute physiological demands of football match play and training across multiple codes (Castagna et al., 2007; Hill-Haas, Coutts, Rowsell, & Dawson, 2008; Hill-Haas, Dawson, Impellizzeri, & Coutts, 2011; Kennett, Kempton, & Coutts, 2012). Two studies have specifically examined the acute activity profile and physiological demands of recreational SSG (soccer) in untrained cohorts (Krstrup et al., 2010c; Randers et al., 2010b). An acute football (soccer) specific SSG session in untrained men and women was structured as seven-a-side (7 v 7) 1 h training session on a 40 x 60 m natural grass pitch, organised as 12 min quarters, interspersed by 2 min rest periods (Randers et al., 2010b). Results demonstrated a mean HR during the SSG games of 80-85% HR\text{max}, with the HR being >90% HR\text{max} for >15% of the sessions for men and ~10% for women. These elevated HR values were observed even though participants spent ~70% of the game duration standing and walking. An explanation may be due to the locomotor activities recorded during the games does not depict the additional energy demands involved with completing the technical requirements of the games (i.e. turns, dribbles, shots, backwards running). Furthermore, these HR responses are similar to Krstrup et al. (2010d) who also reported that over a 1 h session of 7v7 soccer the total number of activity changes was 886 ±44, corresponding to a change in activity every ~4 s. A total of 16 ±1 sprints (efforts >25 km\text{h}^{-1}) and 98 ±5 high intensity runs (>15 km\text{h}^{-1}) were performed with a mean duration of 1.9 ±0.1 and 2.3 ±0.1 s, respectively (Krstrup et al., 2010d). These data further reflected in the HR zones, which showed a mean of 82% HR\text{max} with 20% of the time spent above 90% HR\text{max}. Collectively, these results demonstrate the significant cardiovascular strain (represented through HR indices) in response to an acute SSG session and provides evidence of a marked physiological response in an untrained cohort.
A repercussion of the significant cardiovascular strain induced during an acute SSG session is the subsequent increases in metabolic activity, which is reflected in systemic and skeletal muscle metabolites. Specifically, an increased blood glucose (peak 5.5 ±0.5 and 3.3 ±0.4 mmol L⁻¹ at 30 min into SSG and running, respectively) and lactate (5.2 ±0.6 and 3.7 ±0.4 mmol L⁻¹ 30 min after) concentrations during and after a SSG session shows distinct differences when compared to a continuous treadmill running session, which was matched to induce a similar mean HRmax as SSG (82 ±2% and 82 ±1% HRmax for SSG and continuous running, respectively) (Krustrup et al., 2010d). These results are further reflected in skeletal muscle metabolites, lactate, creatine phosphate and glycogen. Specifically, at the end of the sessions, muscle lactate was higher in SSG (30.1 ±4.1 mmol kg⁻¹ d.w) than in continuous running (15.6 ±3.3 mmol kg⁻¹ d.w) (Krustrup et al., 2010d). Muscle creatine phosphate in SSG and running was 37 and 30% lower, respectively (p<0.05) and muscle glycogen decreased (p<0.05) in both conditions by 118 ±27 and 100 ±33 mmol kg⁻¹ d.w, respectively (Krustrup et al., 2010d). Collectively, these responses to a single bout of SSG and running (performed 4 weeks into a 12-week training period) showed that the SSG session had significantly higher concentrations of blood and muscle lactate as well as blood glucose compared to treadmill running (Krustrup et al., 2010b). Whilst the acute physiological requirements and changes in blood and muscle metabolites provide important insight into the physiological strain of exercise, they do not provide information regarding the inflammatory response or glucose regulation. This information is required to assess the efficacy of SSGs as a suitable training mode for preventing and treating individuals with cardiovascular and metabolic diseases. This has the potential to assist in developing alternative exercise prescription models for both Indigenous and Caucasian communities as a means to increases levels of physical activity and prevent the onset of chronic disease/s such as CVD and T2DM.
2.8.2: Training adaptations to small-sided games in untrained populations

Aerobic capacity

Training intensity is an important factor for reversing risk-factors associated with the development of metabolic abnormalities (Tjonna et al., 2008). High-intensity interval training has been reported to be more effective at improving VO$_{2\text{max}}$ when compared to continuous moderate intensity exercise (Nybo et al., 2010). Moreover, the aerobic capacity adaptation to short-term soccer training has shown to be greater (Meyer, Auracher, Heeg, Urhausen, & Kindermann, 2007) or equal to (Bangsbo et al., 2010) training a volume-matched continuous running programs, and similar to the effects of HIIT (running) (Nybo et al., 2010). Additionally, sedentary men who completed 12 weeks of SSG training increased VO$_{2\text{max}}$ by 13% and 8% in the running condition (Krstrup et al., 2010b). In particular, this study reported a comparable increase in VO$_{2\text{max}}$ between SSG (7%) and continuous running (6%) after the first 4 weeks of training, which increased a further 6% from weeks 4 to 12 only in the SSG condition. These results suggest that the cardiovascular adaptations are similar between conditions in the initial 4 weeks. Providing the matching of training load and progression between conditions these results may also suggest that SSG training may be more effective compared to running over the 12 week duration of the study. Moreover, 16 weeks of training in premenopausal women showed SSG and running training to increase VO$_{2\text{max}}$ by 7% and 6% in the first 4 weeks, respectively (Bangsbo et al., 2010). Conversely, similar increases were still evident between conditions after 16 weeks, by which time a further improvement of 8% for SSG and 4% for running groups were observed (Bangsbo et al., 2010). Correspondingly, after the 16 week training program, time to exhaustion in a GXT increased by 21% in SSG and 16% in running groups (Bangsbo et al., 2010). Taken collectively, SSG appears to be effective in stimulating cardiovascular adaptations, which may be a consequence of both aerobic and anaerobic energy turnover during SSG. Hence,
soccer specific SSG appears to be an effective mode of training in untrained men leading to significant improvements in aerobic capacity that are either comparable or better than continuous running (Bangsbo et al., 2010; Krstrup et al., 2010b).

**Blood pressure**

Two studies examining changes in blood pressure following SSG training in a hypertensive cohort, reported 12-weeks of SSG training similarly improved systolic and diastolic blood pressure with a range of decline of 8-7.5 and 5-10.3 mmHg, respectively (Knoepfl-Lenzin et al., 2010; Andersen et al., 2010; Krstrup et al., 2013). When matched for training volume and intensity, similar decreases (range of decline 7-5.9 and 5-6.9 mmHg for systolic and diastolic, respectively) were also evident in the running condition (Knoepfl-Lenzin et al., 2010; Andersen et al., 2010; Krstrup et al., 2013). The lowered blood pressure was associated with a reduction in resting heart rate, which may reflect reduced sympathetic outflow and thus reduced vascular resistance (Andersen et al., 2010b). Notably, a meta-analysis with endurance exercise interventions of at least 4 weeks showed a more pronounced response in hypertensive (6.9/4.9 mmHg, systolic/diastolic) when compared to normotensive (1.9/1.6 mmHg, systolic/diastolic) cohorts (Cornelissen & Fagard, 2005).

Moreover, in normotensive pre-menopausal women, 16-weeks of SSG and running in resulted in significant changes in cardiac dimensions and had favourable effects of both left ventricular systolic and diastolic function. These differences between conditions were significant for left ventricular posterior wall thickness and isovolumetric relaxation time (Andersen et al., 2010a). These findings are further supported by a more recent study that demonstrated a decrease in blood pressure (-12 ±3 and -6 ±2 mmHg in systolic and diastolic values, respectively) after 15 weeks of SSG (futsal) training in middle-aged hypertensive women (Mohr et al., 2014). Collectively, these results indicate that SSG training in untrained

BODY COMPOSITION

WHEN FOCUSING ON ADAPTATIONS OF BODY COMPOSITION A RECENT STUDY COMPARING SSG WITH CONTINUOUS RUNNING (12-16 WEEKS, 60 MIN SESSIONS, 2-3 D\WK\(^1\)) FOR UNTRAINED MEN (N=36, AGED 20-43 Y) RESULTED IN A LOWERED FAT-MASS AND AN INCREASE IN TOTAL AND LEG LEAN MASS (KRISTRUP ET AL., 2010C). SPECIFICALLY, BODY MASS WAS REDUCED BY 1.1 AND 1.0 KG (P<0.05) IN RESPONSE TO SSG AND RUNNING TRAINING, RESPECTIVELY. FURTHERMORE, TOTAL BODY FAT-MASS (TB-FM) AND FAT PERCENTAGE REDUCED BY 2.7 KG AND 3.0% IN THE SSG CONDITIONS (P<0.05) AND COMPARETIVELY DECREASED IN THE RUNNING CONDITION BY 1.8 KG AND 1.8% (P<0.05) (KRISTRUP ET AL., 2009). IN THE SSG CONDITION, TOTAL BODY FAT-FREE MASS (TB-FFM) WAS 1.7 KG HIGHER AFTER 12 WEEKS OF TRAINING (P<0.05), WITH NO SIGNIFICANT CHANGES REPORTED IN THE RUNNING OR CONTROL CONDITIONS (P>0.05). IN ADDITION, 16 MONTHS OF SSG AND RUNNING TRAINING IN PREMENOPAUSAL WOMEN DEMONSTRATED A SIGNIFICANT INCREASE IN LOWER EXTREMITY FAT-FREE MASS, WITH AN INCREASE OF 1.5 KG IN SSG AND 1.2 KG IN CONTINUOUS RUNNING (BANGSBO ET AL., 2010). A CONSIDERATION WITH THESE STUDIES IS THAT LEG STRENGTH WAS NOT MEASURED IN CONJUNCTION WITH AN INCREASE IN MUSCLE MASS, AS PREVIOUS RESEARCH SUGGESTS 14 WEEKS OF SSG TRAINING IN PREMENOPAUSAL WOMEN INCREASING MAXIMAL DYNAMIC LEG STRENGTH (HELGE ET AL., 2010). NOTABLY, THESE WHOLE-BODY MUSCLE HYPERTROPHIC EFFECTS ARE EQUIVALENT TO RESULTS OBTAINED FROM STRENGTH TRAINING AND GREATER THAN FOR CONTINUOUS RUNNING AND INTERVAL RUNNING (BROEDER, BURRHS, SVANEVICK, VOLPE, & WILMORE, 1997; KRAEMER ET AL., 1999; KRISTRUP ET AL., 2010A).
There are two gender specific studies that report peripheral muscular adaptations in response of SSG and running training (Bangsbo et al., 2010; Krstrup et al., 2010b). Firstly, a 12 weeks training study in untrained men reported the SSG condition to increase in muscle fibre area (15%), estimated quadriceps muscle mass (9%), and a decrease in fraction of type IIx muscle fibers (7.2%), compared to no significant changes in running or control conditions (Krustrup et al., 2010b). Furthermore, the number of capillaries per fibre was 22% and 16% higher following respective SSG and running training programs, respectively (Krustrup et al., 2010b). However, when muscle capillarisation was expressed as capillaries per area, there were no significant changes in SSG (12%) or running (10%) training. Notably, key metabolic enzyme citrate synthase activity increased by 10% and 14% after 4 and 12 weeks of training, respectively, but was unaltered in running and control conditions (Krustrup et al., 2010b). Additionally, a 16 week training study in sedentary reported both SSG and running to increase leg muscle mass and elevated citrate synthase activity after 4 weeks, with no further improvements during the following 12 weeks (Bangsbo et al., 2010). These results demonstrate that in addition to increases in TB-FFM and strength, SSG training is an appropriate stimulus to increase capillarisation, alter fibre type fractions and increase the metabolic activity of the muscle as measured through citrate synthase.

**Blood markers of cholesterol, glucose and CRP**

A loss of fat-mass after exercise training has shown to result in favourable changes in plasma lipids and lipoproteins (Durstine et al., 2001; Kodama et al., 2007; Kokkinos et al., 1995). Moreover, after 12 weeks of SSG training in a previously inactive cohort reported a reduction in fat-mass along with low density lipoproteins (LDL) to HDL-cholesterol ratio, mainly due to the significant decrease in LDL (2.7 to 2.3 mmol·L⁻¹) (Krupstrup et al., 2009). Furthermore, LDL has been reported to decrease in response to an increase in the distance covered per
week along with corollary reductions in body fat (Kokkinos & Fernhall, 1999; Wood et al., 1983). In support, a 12 week study of SSG training in homeless men reported a decrease in TB-FM (2.4%) and an increase in VO$_{2\text{max}}$ occurring in coordination with a decrease of 0.4 mmolL$^{-1}$ LDL and no change in HDL, total cholesterol or triglycerides (Randers et al., 2012). However, SSG training in habitually active men with mild hypertension (25-45 y) demonstrated a reduction in total cholesterol (5.2%) and ratio of total:HDL (10.4%) (Knoepfli-Lenzin et al., 2010). In comparison, changes in cholesterol were not observed in the running condition (Knoepfli-Lenzin et al., 2010). Although speculative, the inconsistent outcomes relating to cholesterol may be related to the different baseline characteristics of the participant cohort. Regardless, SSG training has shown to be more effective at reducing cholesterol when compared to continuous running, which justifies a decrease in risk associated with the future development of CVD.

Several studies have assessed the glucose response to SSG training in untrained cohorts (Krustrup et al., 2010d; Krustrup et al., 2010e; Randers et al., 2010a; Randers et al., 2012), with only one study assessing changes in CRP concentration (Andersen et al., 2010b). In particular, 12 weeks of SSG training in homeless men showed no change in resting glucose (4.9 to 5.0 mmolL$^{-1}$) and insulin (36 to 41 pmolL$^{-1}$) concentrations (Randers et al., 2012). Such findings are consistent across many studies which report no change in fasting glucose and glucose response 2 h post OGTT following 12-weeks to 12 months of either SSG or running training also report (Krustrup et al., 2010d; Krustrup et al., 2010e; Randers et al., 2010a). Specifically, the authors acknowledge that the participants were within normal resting glucose and insulin indices, thus, the scope for these values to further decrease is minimal and not anticipated (Randers et al., 2010a). While these measures have clinical relevance, it would be of interest to measure insulin and glucose throughout the 2 h OGTT (0,
30, 60, 90 and 120 min post glucose load) to provide a detailed description of changes in
time-course metabolism associated with SSG training, although this detailed approach to SSG
has not yet been conducted (Kitabchi et al., 2013).

It must be noted that the wider literature reports inconsistent findings in regards to exercise
training induced effects on CRP, with reductions demonstrating to be dependent or
independent to changes in fat-mass (Donges et al., 2010; You et al., 2013). One previous
study has reported that soccer-specific training in hypertensive men decrease total body,
gynoid and android fat-mass without any change in CRP concentration (Andersen et al.,
2010b). A potential explanation for this may extend to the clinically hypertensive cohort used
in that study, which was inclusive of smokers (n=5), and those on medications (statins, n=2
and anti-hypertensive medications, n=15), both of which can influence CRP concentration
independent of exercise (Schaefer et al., 2005). Accordingly, while there is a distinct lack of
literature specific to inflammatory adaptations to SSG training there is also no literature on
healthy, inactive populations. Small-sided games have the ability to change variables of
fitness and body composition in these healthy cohorts, thus, given the influence of fat mass
and muscle mass on changes in the chronic inflammatory state, the scope for subsequent
adaptations in inflammatory markers is plausible.

An acute SSG bout is a stimulus capable of inducing substantial physiological strain and
energy metabolism, as shown in skeletal muscle and blood. Additionally, when comparing
the metabolite responses of SSG to a continuous treadmill running condition (matched for
mean HR_{max}), results demonstrated distinct differences in glucose (blood) and lactate (blood
and skeletal muscle) responses, which may be a consequence of the transient high-intensity
bouts incorporated within the SSG condition (Holloszy, Kohrt, & Hansen, 1998).
Furthermore, SSG as an acute stimulus is applied over a short-term training period leads to significant musculoskeletal, metabolic and cardiovascular adaptations that are of importance for preventing the onset of metabolic and cardiovascular abnormalities. For these reasons football SSGs may be an achievable panacea for low levels of physical activity within both Indigenous and non-Indigenous communities. However, to date there is minimal research on the physiological health benefits of using football, particularly rugby, as a mode to improve inflammatory and glucose-regulatory risk-factors associated with the development of T2DM and CVD in inactive cohorts. For these reasons, this thesis will focus on the application of SSG as a potential model for exercise prescription in physically inactivity communities (Indigenous and Caucasian men). In particular, SSG may provide a substantial physiological stimulus that is better than or equal too more traditional exercise modes predominantly focusing on one training component i.e. moderate intensity continuous aerobic exercise applied as cycle ergometry.

2.8.3: Manipulating external load to influence intensity during small-sided games

The physiological demands of match play and training have been widely reported from many football codes, with numerous studies investigating different aspects of the games such as activity profile, physiological demands and physical loading (Bangsbo, Nørregaard, & Thorsoe, 1991; Deutsch, Kearney, & Rehrer, 2007; Hill-Haas et al., 2011; Kennett et al., 2012; Stølen, Chamari, Castagna, & Wisløff, 2005). However, most of these studies are specific to the trained and athlete populations, with one study previously examining the physiological demands of recreational football (soccer) in untrained cohorts (Castagna et al., 2007; Randers et al., 2010b). Nonetheless, it has been consistently reported that the perceptual (i.e. Rating of Perceived Exertion, RPE) and physiological load (HR and blood lactate) during soccer specific SSG can be controlled through the manipulation of external
load such as field size, player number, rule modification and coach encouragement (Hill-Haas et al., 2011; Rampinini, Impellizzeri, & Castagna, 2007). Previously, SSG were used to develop technical and tactical abilities in skilled players; however, they are now used by many amateur teams (trained/untrained) as an effective tool for aerobic training, capable of improving cardiovascular and musculoskeletal health (Krustrup et al., 2009; Rampinini et al., 2007) and have been shown to meet the ACSM guidelines for appropriate exercise intensity for increasing aerobic capacity (Garber et al., 2011).

It is common to reduce both the number of players and the size of the playing field to increase the activity profile of SSGs (Kennett et al., 2012; Rampinini et al., 2007). One particular study organised untrained men to complete three sessions of 7 v 7. Each session was 60 min on a 40 x 60 m natural grass pitch, organised as 4 x 12 min exercise periods interspersed with 2 min rest periods. A further 8 players also completed a 3v3 and a 1v1 (5 x 6 min exercise periods, interspersed with 2 min recovery) training sessions (Randers et al., 2010b). Results showed no difference in mean HR in response to player numbers (7 v 7, 3 v 3, and 1 v 1), although the activity profile increased with a decrease in participant numbers (Randers et al., 2010b). These differences in the activity patterns included the mean duration (s) of high-intensity runs, low intensity actions, sprinting (25 km h⁻¹), high speed running (18 km h⁻¹) and medium-speed running (15 km h⁻¹). For an untrained population this factor becomes largely important knowing that by increasing the activity profile (i.e. number of high intensity sprints) of the game can increase the chance of sustaining a musculoskeletal injury (Warburton, Nicol, & Bredin, 2006b). For this reason alone, when prescribing SSG to an inactive or sedentary untrained cohort it is suggested that larger participant numbers (i.e. 5 v 5, 6 v 6 or 7 v 7) are utilised, whilst still stimulating a suitable physiological and perceptual training load.
Previous reports have demonstrated that the physiological and perceptual load can be manipulated through player number and field size (Hill-Haas et al., 2011). Furthermore, it has also been suggested that the field size can be manipulated to modify the intensity of the game. There is a higher activity profile, HR, lactate and RPE when SSG is played on a large field size (i.e. 36 x 48 m) compared to on a smaller field (i.e. 24 x 32 m) (Casamichana & Castellano, 2010; Rampinini et al., 2007). Although, another study reported no significant differences in the HR response between large (30 x 20 m) and small field (50 x 40m) sizes (Kelly & Drust, 2009). Accordingly, the majority of literature supports a greater physiological demand when SSG is conducted on a larger field size. However, these studies are specific to soccer SSG and more recently additional interest has been directed towards SSG across other football codes such as rugby.

A recent study in semi-professional rugby players reported the physiological response to a series of SSG of varying player numbers (4 v 4, 6 v 6, and 8 v 8) on small- (32 x 24 m) and larger-sized fields (64 x 48 m) (Kennett et al., 2012). Kennett et al. (2012) demonstrated differences between all player numbers and field sizes in mean speed (m·min⁻¹), high-speed running, distance covered (m), RPE and blood lactate concentration, with no differences in the HR responses (% HRmax and time spent >85% HRmax). Similar to the results previously reported in soccer specific SSG, this study showed that fewer player numbers on a larger field size elicits greater physiological and perceptual responses and time motion demand (Kennett et al., 2012; Randers et al., 2010b). Accordingly, no change in HR with player number or field size is in contrast to previous soccer specific reports (Casamichana & Castellano, 2010; Rampinini et al., 2007) and in support of others (Kelly & Drust, 2009). Notably, coach encouragement throughout the training session has also shown to elicit a higher perceptual and physiological intensity (i.e. HR, blood lactate and RPE) when compared to no
encouragement (Rampinini et al., 2007). Collectively, similar results across football codes indicated that time motion characteristics and RPE can be controlled by manipulating field size, player numbers and coach encouragement, albeit no data is available specific to an untrained cohort between football codes, thus care should be taken for the use of these results within an inactive, untrained population.

2.8.4: Reproducibility of small-sided games

Although knowledge of exercise intensity within SSG is useful, the reproducibility and inter-participant variability is an important factor to verify whether the physiological strain is consistent between players and therefore be confidently applied to provide a similar stimulus in a group training environment (Rampinini et al., 2007). Hill-Haas et al. (2008) reported that in a 6 v 6 SSG session (soccer; field size 49 x 37 m), HR_{max}, peak HR, mean sprint duration (s) and mean sprint distance (m) had a typical error variation of 3.4%, 3.6%, 13.0% and 14.5%, respectively. Moreover, the variability for coach encouragement during a 6 v 6 SSG (soccer) session were assessed on field dimensions small (24 x 32 m), medium (30 x 40 m) and large (36 x 48 m) (Rampinini et al., 2007). Results highlighted the co-efficient of variance (CV%) for HR_{max} on fields of small, medium and large sizes were 2.2%, 2.6%, and 2.7%, with an intra-participant variability of 4.7%, 3.6% and 4.8%, respectively (Rampinini et al., 2007). Additionally, RPE variability on field’s small, medium and large were 9.0%, 8.7% and 11.3%, and intra-participant variability were 16.9%, 12.6% and 16.6%, respectively (Rampinini et al., 2007). Again these results are specific to soccer SSG, with rugby specific SSG also reporting high reproducibility for the movement demands of each SSG when repeated (total distance: ICC (±90% confidence intervals): r=0.90 (0.82-0.95), mean speed r=0.87 (0.76-0.093) and high speed running (14.5-23 km h^{-1}) r=0.90 (0.82-0.95) (Kennett et al., 2012). Taken collectively, SSG applied across football codes (soccer and rugby) is highly
reproducible and can be utilised as an intervention that is capable of manipulating and stimulating physiological and perceptual responses (Impellizzeri et al., 2006; Kennett et al., 2012; Rampinini et al., 2007). Furthermore, the use of rugby specific SSG is of interest for this thesis and as demonstrated by Kennett et al. (2012) has comparable physiological demands as soccer-specific SSG.

2.8.5: Matching training load between intermittent and continuous conditions

Research is required on rugby specific SSG as a stimulus that competes with traditional continuous exercise modes such as cycle ergometry. The ability to measure, monitor and manipulate exercise intensity is essential when comparing between exercise modes. Common measures of intensity are based on perceptual (i.e. RPE) and physiological strain (i.e. HR and blood lactate) (Borg, 1982; Hill-Haas, Coutts, Rowsell, & Dawson, 2009). It has been suggested that the use of both HR and lactate during a SSG session is better related to RPE than either measure alone (Coutts, Rampinini, Marcora, Castagna, & Impellizzeri, 2009). Accordingly, all three variables should be measured to ensure consistent and reliable monitoring of intensity, especially when comparing across exercise modes.

To date, research has concentrated on the effects of prolonged continuous exercise because it is easily controlled and manipulated. Moreover, when assessing the training load during SSG there are inherent problems when trying to monitor the non-uniform movement patterns involving multiple and irregular sprints, interspersed by a high volume of standing or walking. These problems are partially overcome through the use of global positioning system (GPS) devices, which has shown acceptable reliability and validity when monitoring across multiple movement patterns and speeds; providing the GPS systems are not used interchangeably (Coutts & Duffield, 2010). These GPS systems assist with monitoring the
activity profile of the SSG games so external loading factors (i.e. field size and player numbers) can be manipulated to achieve an intensity based on the collective analysis of HR, lactate and RPE variables. Whilst it is acknowledged that there are inherent problems relating to matching of external load between SSG and cycle ergometry, both conditions can have their respective external load manipulated to achieve a particular intensity based off the monitoring of perceptual and physiological strain. Thus, despite the obvious difficulties in standardising external load and environmental factors the reproducibility of intensity in SSG compares well with cycle ergometry within a laboratory or gymnasium environment.

2.9: Summary of literature review

Numerous studies demonstrate that continuous aerobic exercise modes have the capabilities to induce decreases in pro-inflammatory cytokines and subsequently reduce the risk associated with the onset of chronic disease development (Aronson et al., 2004a; Panagiotakos et al., 2005). Accordingly, there is increasing interest in the application of exercise interventions to reduce the chronic inflammatory state and disease risk (Beavers et al., 2010; Donges et al., 2010; Lavie et al., 2011; Nicklas et al., 2005). Finally, the repercussions of an improved inflammatory state have direct consequences on glycaemic control and insulin signalling, which collectively form as risk-factors associated with the development of metabolic and cardiovascular abnormalities (Arend, 2002; Hotamisligil, 2006; Ouchi et al., 2011). Skeletal muscle is the major site for glucose disposal in lean healthy glucose tolerant individuals (Defronzo et al., 1981b; Holloszy & Coyle, 1984; Kristiansen et al., 2000). The exact mechanism that leads to the development of insulin resistance in skeletal muscle is not fully understood. However, it is proposed that a combination of factors associated with systemic inflammation and defects in mitochondrial
functioning collectively contribute to reduced insulin release, insulin sensitivity and signalling within the muscle (Egan & Zierath, 2013; Finck & Kelly, 2006; Kim et al., 2008; Lira et al., 2010). Given the role of mitochondria in regulating cellular glucose uptake and providing energy balance, positive mitochondrial adaptations to exercise may provide a method to improve glucose tolerance (Hoppeler & Fluck, 2003). As such, the preservation of aerobic capacity and skeletal muscle strength through exercise training can ameliorate metabolic abnormalities, potentially through the extensive molecular remodelling of the skeletal muscle mitochondria (Egan & Zierath, 2013; Hoppeler & Fluck, 2003).

Indeed, the benefits of exercise for chronic disease prevention have traditionally been associated with systemic adaptations and improvements in clinical risk factors (i.e. fat-mass, muscle mass, inflammatory state, aerobic capacity, lipid profile) associated with glucose regulation and/or insulin sensitivity (Alberti et al., 2006; Durstine et al., 2012; Laaksonen et al., 2002). In part, these clinical benefits have been attributed to metabolic and mitochondrial remodelling within skeletal muscle (Egan & Zierath, 2013). To date, the majority of research has focused on these respective clinical health benefits in relation to continuous, aerobic-based exercise training, such as cycle ergometry (Egan & Zierath, 2013; Garber et al., 2011). Such exercise stimuli predominately involves lower-body, concentric muscular contractions capable of reducing fat-mass, and improving the chronic inflammatory state, aerobic capacity, glucose regulation and mitochondrial biogenesis (Balducci et al., 2010; Bijker et al., 2002; Donges et al., 2010; Egan & Zierath, 2013; Goodyear & Kahn, 1998; Hawley & Lessard, 2008; Irrcher et al., 2003; Libardi et al., 2012). However, despite a clear lack of evidence, it is acknowledged that interval-based training can result in similar or greater adaptations in aerobic capacity, glucose regulation, mitochondrial activity and pro-
inflammatory states, when compared to continuous exercise in healthy adults (Croft et al., 2009; Gormley et al., 2008; Helgerud et al., 2007; Nybo et al., 2010; Whyte et al., 2010).

A common difficulty within the Indigenous Australian population is the lack of facilities to promote the conventional approach to exercise training through gym-specific methods of aerobic (i.e. cycle ergometry or running) and resistance-based exercises. These types of structured exercise training programs provide physiological benefits specific to inflammation, glucose regulation and body composition. However, the limitation with implementing these conventional programs within regional Indigenous Australian populations is that it does not represent the communities’ physical activity patterns, interest and/or understanding of community involvement into one’s health. In particular, Australian football and rugby league is of highest popularity amongst Indigenous Australians (Neesham & Garnham, 2012), and thus creates an opportunity for encouraging community engagement and ownership over an exercise program. However, as yet this sports-specific approach to exercise training in Indigenous Australians does not exist. Thus investigating this method of exercise prescription may provide an under researched populations with high rates of physical inactivity, obesity and chronic disease with an opportunity of evidence-based research for exercise prescription, specific to their cultural and community needs. Not only that, the notion of SSG represents a concurrent inclusion of the virtues of both high-intensity intermittent like exercise over prolonged durations reminiscent of continuous endurance exercise – ideally in a palatable form to ensure sufficient adherence.

Furthermore, SSG’s have an added benefit of providing a socially-inclusive approach to increasing levels of physical activity and provides an additional method of prescription for those without access to specialised equipment and/or those impartial to gym-based training
Accordingly, soccer specific SSG training has been of recent research interest in inactive Caucasian men and women, which has shown positive functional (i.e. cardiopulmonary and strength), body composition (i.e. increased muscle mass and decrease fat-mass) and skeletal muscle (i.e. increased capillary density, fibre type and metabolic efficiency) adaptations (Krustrup et al., 2010a; Krustrup et al., 2010b; Krustrup et al., 2009). However, no data is available specific to systemic inflammatory improvements and/or systemic and skeletal muscle glucose regulatory adaptations within inactive, middle-aged men.

The potential health benefits resultant from both cycle ergometry and SSG training across inactive populations include the acute responses in glucose utilisation and the release of anti-inflammatory cytokines. It is assumed the regularity of this acute stress via exercise training promotes improved chronic adaption in mitochondrial biogenesis, glucose regulation, body composition and systemic inflammation (Gleeson, 2013; Lanza & Sreekumaran Nair, 2010). However, whether SSG can provide additional improvements for the aforementioned parameters relating to physical health remain to be fully elucidated. Further, no literature currently outlines the respective acute demands of SSG within inactive populations compared to traditional laboratory based modes such as cycle ergometry. Moreover, despite high rates of chronic disease, obesity and physical inactivity no acute or chronic exercise programs have been assessed within Indigenous Australian men.
CHAPTER THREE

Differences in post-exercise inflammatory and glucose responses between inactive Indigenous Australian and Caucasian men completing a single bout of cycle ergometry.

As accepted for publication

3.1: Abstract

Aim: This study compared the acute inflammatory and glucose responses following aerobic exercise in sedentary Indigenous Australian and Caucasian men, matched for aerobic capacity and body composition.

Methods: Sedentary Indigenous (n=10) and Caucasian (n=9) Australian men who were free from chronic disease volunteered to participate. Following baseline testing participants completed a 40 min cycle ergometry (CYC) bout at ~80% HRmax. Fasting venous blood was collected pre, 0, 30, 60 and 240 min post-exercise for analysis of glucose, insulin, cortisol, TNF-α, IL-1β, IL-6, IL-1ra and CRP.

Results: Resting TNF-α and glucose concentrations were significantly higher in the Indigenous group (p<0.05). IL-6 and IL-1ra were elevated for longer in Caucasian (p<0.05), compared to the Indigenous group (p>0.05). The post-exercise (0 min) increase in cortisol and glucose for the Caucasians was higher (p<0.05) than the attenuated responses within the Indigenous group (p>0.05).

Conclusion: Despite being matched for aerobic capacity and body composition the Indigenous men had elevated resting TNF-α and glucose compared to the Caucasian men, which may have contributed to the suppressed post-exercise anti-inflammatory response of the Indigenous men; however, glucose normalised between groups post-exercise. As such, it is recommended for acute moderate-intensity exercise to be completed daily for long-term improvements in glucose regulation, irrespective of ancestry. Of note, results suggest it to be even more pertinent for exercise to be encouraged within the Indigenous Australian population due to their elevated resting glucose levels at a younger aged, when compared to the respective Caucasian group.
3.2: Introduction

A comparison of physiological characteristics among different ancestral populations can provide insight into the disparity in health status between population groups (Miller & Cappuccio, 2007; Thorburn, Brand, O’dea, Spargo, & Truswell, 1987). The worldwide incidence rates of T2DM has been rising for the past 30 y with differences evident between population groups that are specific to ethnicity, geographical location and socioeconomic status (Albert, 2007; Cleland & Sattar, 2005; Naqshbandi et al., 2008). Many Indigenous groups experience a lower life expectancy consequent to increased prevalence of non-communicable chronic diseases (Albert, 2007; Australian Institute of Health and Welfare, 2011; Cleland & Sattar, 2005). A prominent example of population-based differences is observed between Indigenous Australians and their Caucasian counterparts (Australian Institute of Health and Welfare, 2011; Thorburn et al., 1987). As an illustration of this disparity, the age-standardised rate for Indigenous Australians with diabetes is estimated at 12%, compared with 4% for non-Indigenous Australians (Australian Institute of Health and Welfare, 2011; Thomson et al., 2011; Trewin & Madden, 2005). Despite the higher prevalence of diabetes amongst Indigenous Australians, there is little published research reporting the acute physiological benefits of physical activity, which is known to be an essential component for reducing the potential disease risk in the long-term.

Physical inactivity is well known to influence inflammatory and glucose regulatory mechanisms that contribute to the development of T2DM and CVD (Kahn et al., 2006; Pradhan et al., 2001). An inverse relationship has been shown between physical activity, excess fat mass and systemic inflammatory biomarkers, which are predictors of all-cause mortality (Koenig, Khuseyinova, Baumert, & Meisinger, 2008). Therefore, regular physical activity may be an important factor in eliciting favourable acute inflammatory and glucose
regulatory responses that positively impact on these parameters (Colberg, 2007; Rowley et al., 2000a).

The clarification of differences in metabolic and inflammatory parameters enhanced by physical activity would be important in establishing whether the disparity in resting metabolic health between Indigenous and Caucasian Australians could be positively altered by an acute exercise bout. Therefore, the aim of this study was to compare the resting and exercise-induced inflammatory and glucose responses of an acute standardised bout of aerobic exercise in middle-aged, sedentary Indigenous Australian and Caucasian men, matched for body composition and aerobic capacity.

3.3: Methods

3.3.1: Participant recruitment

Men who self-identified to have Australian Indigenous (with at least one parent of Aboriginal descent) (n=10) or Caucasian (n=9) ancestry were recruited from a regional New South Wales community. Participants were additionally matched for aerobic capacity and body composition (Table 3.1). Prior to data collection, verbal and written consent was provided followed by the completion of a pre-exercise health questionnaire and the Medical Outcomes Study Short-Form Health Survey (MOS SF-36). The life-expectancy of Indigenous and non-Indigenous Australian men is 67.2 y and 78.7 y, respectively (Australian Institute of Health and Welfare, 2011). Thus, recruitment ensured participants whom represented their middle-aged cohort allowing for a ~11.5 y gap between the respective groups (Australian Institute of Health and Welfare, 2011). Participants were sedentary (<1 exercise session per week for <60 min) but not clinically diagnosed with any pre-existing CVD or metabolic disorders.
Exclusion criteria included: Smokers (< 1 y cessation); suffering from recurrent or recent influenza illness (including flu shot recipients); recent surgical patients; those on cholesterol lowering, anti-inflammatory, or any other medication reported to affect the inflammatory response; rheumatoid arthritis; known or recent periodontal disease. Any participant with a resting concentration >10.0 mg·L⁻¹ for CRP was excluded (Pearson et al., 2003). The study conformed to the Declaration of Helsinki and was approved by the Institutional Research in Human Ethics Committee.

3.3.2: Baseline testing

Participants completed two sessions separated by a 7 d recovery period. The first session comprised of baseline testing, which also acted as an information and familiarisation session to explain all details of the study and testing procedures. Anthropometric data were obtained at baseline testing, including stature, body mass, and waist and hip girths using standard techniques (Marfell-Jones, Stewart, & De Ridder, 2012). Manual blood pressure was obtained by aneroid sphygmomanometer and cuff (Welch-Alyn, Arden, North Carolina, USA) expressed as the mean of three measurements after the participant had been seated for 5 min. A supine whole body DXA scan (XR800, Norland, Cooper Surgical Company, USA) was conducted with scanning resolution set at 6.5 x 13.0 mm, and scanning speed was set at 260 mm·s⁻¹. Whole body scans were analysed (Illuminatus DXA, ver. 4.2.0, USA) for TB-FM and intra-abdominal fat mass (IA-FM) (Kim, Wang, Heymsfield, Baumgartner, & Gallagher, 2002). Analysis of IA-FM was performed with the creation of a region of interest standardised across all participants according to previously outlined procedures (Hill, Laforgia, Coates, Buckley, & Howe, 2007).
Aerobic capacity measures were obtained via a GXT to determine sub-maximal VO₂ and power output (Watts). Prior to each test the metabolic gas analysis system (Parvo Medics, True2400, East Sandy, UT, USA) was calibrated and the GXT was performed on an electronically braked cycle ergometer (LODE Excalibur Sport, LODE BV, Groningen, The Netherlands). The test commenced at 25 W and increased by 25 W every min. Participants exercised until attainment of 80% age-predicted (220 – age) HRₘₐₓ to minimise the risks associated with maximal physical capacity in potentially higher-risk participants. The last stage completed during the GXT test was converted from watts (W) into kilopond (kp) (Colton & Larsen, 2002) and used as the exercise intensity for a cadence of 60-65 rpm for the following CYC session.

3.3.3: Exercise protocol

Following an overnight fast (10-12 h), participants arrived at the laboratory (between 0600 and 0800 h) and completed an acute bout of CYC. During all testing procedures the laboratory was at a controlled temperature of 18-20°C. Participants refrained from any physical activity 48 h prior and alcohol and/or caffeine consumption 24 h prior to testing. During exercise and for 240 min after all testing sessions, participants remained fasted and consumed water *ab libitum* (~500 mL). The cycling session was conducted on a stationary cycle ergometer (Monark 828E, Varburg, Sweden) and comprised of 4 x 10 min efforts, at an intensity of 80% HRₘₐₓ, interspersed by 2 min passive recovery. Participants maintained a cadence of 60-65 revolutions per minute (rpm) with baseline resistance set at the last completed workload of the GXT and (if required) manipulated to maintain the target intensity. Heart rate was monitored throughout (Vantage NV, Polar, Finland) and RPE (Borg’s 6-20 scale) were recorded as a session-RPE 30 min post-exercise (Herman, Foster, Maher, Mikat, & Porcari, 2006). Of note, whilst the current study only involved the participants completing
one exercise session, the methodology was designed to match an aerobic exercise prescription approach for a sedentary population (Garber et al., 2011).

3.3.4: Venous blood collection

Prior to the commencement of the protocol, participants were cannulated for the collection of venous blood samples before and immediately (0 min), 30, 60 and 240 min after exercise (Ostrowski et al., 1999). The medial antecubital vein was cannulated and flushed with saline to ensure a clear line. Prior to collecting blood for analysis, saline was drawn from the line in another syringe and discarded. During cannulation and all blood draws, participant postural position was standardised in the up-right position. Serum was collected in a serum separated tube (SST) tube for analysis of lipid profile, CRP, insulin and cortisol. Plasma was collected in an edetate calcium disodium agent (EDTA) tube for the analysis of HbA1c, total leukocyte count, IL-6, IL-1ra, IL-1β and TNF-α; as well as a fluoride oxalate (FO) tube for analysis of glucose. Following the clotting of the sample (SST) or immediately following collection (EDTA, FO) samples were centrifuged at 3500 rpm for 15 min at 4°C. Aliquots were frozen immediately at -80°C and -20°C for EDTA and SST, respectively. Whole blood was refrigerated (4°C) for a maximum of 6 h until analysis of total leukocyte count and HbA1c.

3.3.5: Venous blood analyses

Fasting venous blood were collected at rest for descriptive measures (Maple-Brown et al., 2012) of total cholesterol (Enzymatic method and polychromatic endpoint technique), HDL (Accelerator selective detergent methodology), LDL (Friedwald Equation), triglycerides (Enzymatic method and biochromatic endpoint technique: Dimension Xpand Plus, Siemens Healthcare Diagnostics, Sydney, Australia), total leukocyte count (Cell counter: Cell-Dyn 3200, Abbott Laboratories, Abbott Park, IL, USA) and HbA1c (High-performance liquid
chromatography: Bio-Rad Variant, Bio-Rad Laboratories, Sydney, Australia). For the analysis of glucose regulatory parameters (Pedersen & Febbraio, 2008; Steensberg et al., 2002b), 20 mL was collected at each time point for analysis of glucose (ABL825 Flex Analyser, Radiometer Medical ApS, Bronshoj, Denmark), insulin, cortisol (Solid-phase chemiluminescent enzyme immunometric assay: Immulite 2000, Siemens Healthcare Diagnostics, Los Angeles, CA, USA). Analysis of biochemistry variables glucose, insulin, cortisol and CRP showed intra and inter-assay coefficients of variation between 4.0-7.4%. For the analysis of inflammatory parameters (Ostrowski et al., 1999; Pedersen & Febbraio, 2008), CRP (Particle enhanced turbidimetric immunoassay: Dimension Xpand Plus, Siemens Healthcare Diagnostics, Sydney, Australia), IL-6, IL-1β, IL-1ra and TNF-α were measured at all-time points (Sandwich enzyme immunoassay ELISA: Quantikine, R & D Systems, Minneapolis, MN, USA), with intra and inter-assay coefficients of variation between 4.3-5.6%. As an indication of HOMA-IR was calculated based on (fasting insulin x fasting glucose)/22.5 (Matthews et al., 1985). Of note, haematocrit was not measured and none of the respective variables have been corrected for plasma volume change. This is a potential limitation in the current study; however, procedures were enforced within and between all testing procedures to minimise any potential plasma volume shifts i.e. controlled environmental temperature, up-right posture during venous blood collection and standardised fluid consumption prior to, during and following exercise. (Kargotich, Goodman, Keast, & Morton, 1998).

3.3.6: Statistical analyses
All data are reported as mean ± SEM. Within group analysis for all time-points were assessed by a two-way repeated measures ANOVA (condition x time). Differences between-groups for all time-points were assessed by one-way ANOVA with a Tukey’s correction. Significance was accepted at p<0.05. All data that were not normally distributed were log transformed
prior to analysis (CRP, IL-6, IL-1β, IL-1ra and TNF-α). All statistical analyses were performed using PASW™ for MS-Windows version 17.0 (Statistical Package for the Social Sciences, Chicago, IL, USA).

### 3.4: Results

#### 3.4.1: Resting venous blood chemistry, body composition and aerobic capacity variables

All descriptive measures of anthropometry, DXA (TB-FM%, IA-FM kg), and resting venous blood chemistry are presented in Table 3.1. Both groups were matched for TB-FM% and VO₂ (p>0.05); however, the Indigenous group was significantly younger and showed higher concentrations of fasting glucose and TNF-α (p<0.05) compared to the Caucasians. Aerobic power assessed during baseline testing was reported as the last completed stage (W), which did not differ (p=0.52) between Indigenous (183 ±11 W) and Caucasian groups (172 ±14 W). MOS SF-36 questionnaire results showed no significant difference between groups (p=0.67) with a total of 79.2 ±5% for the Indigenous group and 83.9 ±4% for the Caucasian group.
Table 3.1
Baseline characteristics, body composition and fasting blood chemistry of Indigenous and Caucasian participants. Data reported as Mean ± SEM.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Indigenous (n=10)</th>
<th>Caucasian (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)(^a)</td>
<td>38.5 ± 3.2</td>
<td>48.8 ± 1.7</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>177.7 ± 3.1</td>
<td>177.4 ± 1.8</td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>101.5 ± 7.9</td>
<td>101.6 ± 3.3</td>
</tr>
<tr>
<td>BMI (kg·m(^2))</td>
<td>31.9 ± 2.0</td>
<td>32.3 ± 0.9</td>
</tr>
<tr>
<td>Sub-maximal VO(_2) (mL·kg(^{-1})·min(^{-1}))</td>
<td>30.8 ± 1.7</td>
<td>30.8 ± 1.3</td>
</tr>
<tr>
<td>Systole blood pressure (mmHg)</td>
<td>131 ± 3</td>
<td>131 ± 4</td>
</tr>
<tr>
<td>Diastole blood pressure (mmHg)</td>
<td>84 ± 2</td>
<td>82 ± 1.9</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>103.6 ± 5.9</td>
<td>107.0 ± 2.5</td>
</tr>
<tr>
<td>WHR</td>
<td>0.95 ± 0.03</td>
<td>0.99 ± 0.02</td>
</tr>
<tr>
<td>Total Body -- Fat Mass (%)</td>
<td>27.8 ± 3.6</td>
<td>31.4 ± 1.6</td>
</tr>
<tr>
<td>Intra-Abdominal Fat Mass (kg)</td>
<td>3.29 ± 0.7</td>
<td>3.57 ± 0.29</td>
</tr>
<tr>
<td>Total cholesterol (mmol·L(^{-1}))</td>
<td>5.1 ± 0.3</td>
<td>5.8 ± 0.4</td>
</tr>
<tr>
<td>HDL (mmol·L(^{-1}))</td>
<td>1.1 ± 0.1</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>Triglycerides (mmol·L(^{-1}))</td>
<td>1.6 ± 0.2</td>
<td>2.2 ± 0.4</td>
</tr>
<tr>
<td>Hazard ratio (Total : HDL)</td>
<td>4.8 ± 0.5</td>
<td>4.6 ± 0.4</td>
</tr>
<tr>
<td>HbA1c (%A1c)</td>
<td>5.7 ± 0.2</td>
<td>5.7 ± 0.1</td>
</tr>
<tr>
<td>Glucose (mmol·L(^{-1}))(^a)</td>
<td>5.4 ± 0.2</td>
<td>4.6 ± 0.9</td>
</tr>
<tr>
<td>Insulin (µU·mL(^{-1}))</td>
<td>17.7 ± 4.6</td>
<td>11.5 ± 2.9</td>
</tr>
<tr>
<td>Insulin resistance (HOMA-IR)</td>
<td>4.4 ± 1.2</td>
<td>2.4 ± 0.6</td>
</tr>
<tr>
<td>Total Leukocyte Count (10(^9)·L(^{-1}))</td>
<td>6.9 ± 0.6</td>
<td>6.1 ± 0.4</td>
</tr>
<tr>
<td>Cortisol (nmol·L(^{-1}))</td>
<td>400.8 ± 34.5</td>
<td>463.9 ± 43.6</td>
</tr>
<tr>
<td>CRP (mg·L(^{-1}))</td>
<td>3.3 ± 0.8</td>
<td>3.0 ± 0.7</td>
</tr>
<tr>
<td>IL-6 (pg·mL(^{-1}))</td>
<td>1.0 ± 0.2</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>TNF-α (pg·mL(^{-1}))(^a)</td>
<td>2.6 ± 0.4</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>IL-1ra (pg·mL(^{-1}))</td>
<td>252.6 ± 27.9</td>
<td>235.2 ± 24.3</td>
</tr>
<tr>
<td>IL-1β (pg·mL(^{-1}))</td>
<td>1.42 ± 0.11</td>
<td>1.7 ± 0.1</td>
</tr>
</tbody>
</table>

BMI = Body mass index; VO\(_2\) = Oxygen Consumption; WHR = Waist to hip ratio; HDL = High density lipoprotein; CRP = C-reactive protein; IL = interleukin; ra = receptor agonist; TNF = Tumor necrosis factor.

\(^a\) Significant difference between groups (p<0.05).
3.4.2: Cycle ergometry demands

Resistance for the CYC protocol was not significantly different between groups (p=0.80) at 1.9 ±0.1 kp for Caucasian and 2.0 ±0.3 kp for the Indigenous group. The mean HR response to the CYC protocol was not significantly different (p=0.17) between groups at 81 ±2% for Indigenous and 83 ±1% HRmax for the Caucasian group. Session-RPE was not significantly different between groups (13.9 ±0.5 and 13.6 ±0.4 AU for Indigenous and Caucasian groups, respectively; p=0.54).

3.4.3: Inflammatory response to cycle ergometry

The acute post-exercise responses to CYC for IL-6, IL-1ra, CRP, TNF-α and IL-1β are shown in Figure 3.1. IL-6 exhibited a significant post-exercise increase (p<0.05) for both groups and was not different between groups (p=0.63). However, from 30 to 60 min post-exercise IL-6 significantly decreased within the Indigenous group (p=0.004), whilst the Caucasian group, remained elevated (p=0.27). IL-1ra was significantly elevated post-exercise within both groups (p<0.05), though the Caucasian group showed a sustained elevation of IL-1ra above pre values to 240 min post-exercise (p=0.03) that was not evident in the Indigenous group (p=0.12). Post-exercise CRP and IL-1β responses did not differ within or between groups (p>0.05). No exercise-induced response for TNF-α was evident within either group (p>0.05), whilst between group comparisons indicated the Indigenous group to have significantly higher TNF-α values at all time-points compared to the Caucasian group (p<0.05).
Figure 3.1
Mean ± SEM response of IL-6, IL-1ra, CRP, TNF-α and IL-1β within and between the respective groups. Significant difference from baseline within the Indigenous group \( ^a \) P<0.05; Significant differences from baseline within the Caucasian group \( ^b \) P<0.05; Significant difference between groups \( ^c \) P<0.05.
3.4.3: Cortisol, glucose and insulin response to cycle ergometry

The acute post-exercise response for cortisol, glucose and insulin are shown in Figure 3.2. Cortisol showed a significant increase immediately post-exercise (0 min) within the Caucasian group (p=0.003), but not in the Indigenous group (p=0.15). Between group comparisons showed the Caucasian group to have significantly elevated cortisol values compared to the Indigenous group at 0 (p=0.008), 30 (p=0.014) and 60 min (p=0.002) post-exercise. Glucose responses increased significantly immediately post-exercise within the Caucasian group (p=0.01), but not the Indigenous group (p=0.57). The exercise-induced insulin response showed a pre to 240 min post-exercise decrease within both groups (p<0.05), without significant differences between groups (p=0.39). Furthermore, HOMA-IR values from pre to 240 min post-exercise showed a significant decline within both the Indigenous (53.3 ±7.3%; p=0.007) and Caucasian (42.6 ±5.2%; p=0.03) groups, with no significant differences between groups (p=0.11).
Figure 3.2
Mean ± SEM response of cortisol, glucose and insulin within and between the respective groups.
Significant difference from baseline within the Indigenous group $^a$ P<0.05; Significant differences from baseline within the Caucasian group $^b$ P<0.05; Significant difference between groups $^c$ P<0.05.
3.5: Discussion

The findings demonstrate that despite being matched for aerobic capacity and TB-FM%, Indigenous Australian and Caucasian groups varied in both baseline and post-exercise levels of some plasma inflammatory markers and glucose concentrations. Specifically, the younger Indigenous group had significantly higher fasting concentrations of glucose and TNF-α compared with the Caucasian group. Furthermore, although both groups showed an immediate post-exercise increase in anti-inflammatory markers (IL-6 and IL-1ra), the Caucasian group sustained this elevated anti-inflammatory response longer than the Indigenous group. However, neither group showed any exercise-induced pro-inflammatory response (CRP, IL-1β and TNF-α). Collectively, these findings suggest an acute suppression of anti-inflammatory cytokines to standardised aerobic exercise in sedentary Indigenous men.

A possible limitation in the present study is the recognised difference in chronological age between groups. However, when comparing chronological age between Indigenous and Caucasian populations, life expectancy is significantly different relative to their health status (Thomson et al., 2011). Therefore, matching of age does not provide a fair physiological comparison and can conflate the interpretation of the findings. For this reason the groups in the current study were matched for VO₂ and TB-FM%, as these have a more salient influence on inflammatory markers and glucose regulation, rather than chronological age per se (Arsenault et al., 2009; Church et al., 2002). The present results show that younger, Indigenous men have high fasting glucose concentrations that are subsequently reflected in altered acute post-exercise responses compared to an older Caucasian group. Of concern, the Indigenous group were ~10.2 y younger than the Caucasians group, suggesting that the metabolic health of Indigenous Australians declines at an earlier age than Caucasian Australians. These higher fasting glucose concentrations, if evident over prolonged periods,
can lead to the development of T2DM and associated co-morbidities (Thomson et al., 2011; Trewin & Madden, 2005). Interestingly, on a national scale the Indigenous Australian population have a known susceptibility to developing T2DM and CVD when compared to their Caucasian counterparts (Australian Institute of Health and Welfare, 2011; Thomson et al., 2011; Trewin & Madden, 2005). While data from the current study is from a small sample size, it reiterates these differences in health status between Indigenous and Caucasian Australians. Furthermore, these data also highlight the need for targeted exercise interventions for young Indigenous Australians as a disease preventative approach and also to determine the causative mechanisms that underlie the poorer metabolic health of this group.

Systemic inflammatory biomarkers are highly correlated with increased risk for chronic disease development and all-cause mortality (Koenig et al., 2008; Pradhan et al., 2001). Chronic elevation of TNF-α, IL-6 and IL-1 from adipose tissue stimulates the nuclear factor-kappa beta pathway and regulates the hepatic release of CRP; in turn highlighting a causative role in the development of T2DM and CVD (Kahn et al., 2006). The present study showed no difference between groups in resting concentrations of IL-6, IL-1β or CRP. However, in comparison to the Caucasian group, the Indigenous group had a significantly higher concentration of TNF-α and fasting glucose. Given the strong association of elevated TNF-α with impaired glucose control, higher TNF-α concentrations may provide reason for the observed baseline insulin resistance (>4 HOMA-IR) in the Indigenous group (Pedersen, 2011a). Previous longitudinal studies have compared the health characteristics and effects of chronic exercise training between populations of differing ancestry (Miller & Cappuccio, 2007; Skinner et al., 2001). However, to date no evidence is available involving the acute physiological response to exercise for inflammatory and glucose markers between groups of differing ancestry.
Markers indicative of a pro-inflammatory state (TNF-α, IL-1β, CRP) showed no significant exercise-induced responses; though, TNF-α remained elevated at all time-points in Indigenous compared to the Caucasian group. Moreover, in a rested state, increased IL-6 in the presence of elevated TNF-α is indicative of low-grade chronic systemic inflammation, whilst an exercise-induced increase of IL-6 in the absence of an elevated TNF-α is indicative of increased energy demand (Walsh et al., 2011). Though absent in the present study, an increase in TNF-α has been shown in response to strenuous prolonged exercise, with the magnitude potentially related to exercise duration and associated immunological parameters (Walsh et al., 2011). Notably, CRP is reported to peak 24-48 h post-exercise, and represents one potential limitation of the current data that reports up to 240 min post-exercise (Pedersen & Febbraio, 2008). Despite this limitation, the present study shows that regardless of ancestry, neither group to have exercise-induced increases in pro-inflammatory markers, which may be attributable to the short duration, moderate intensity and/or the interval nature of the condition.

Pro-inflammatory cytokines have potential to be mediated by anti-inflammatory cytokines and cytokine inhibitors (i.e. cortisol), which can increase markedly in the circulation following prolonged exercise (Fischer, 2006; Pedersen, 2011a). Additionally, during strenuous exercise IL-6 has anti-inflammatory properties mediating skeletal muscle signalling processes via the activated protein kinase pathway (i.e. AMPK) and stimulating the expression of other anti-inflammatory cytokines, such as IL-1ra and IL-10 (Pedersen, 2011a; Walsh et al., 2011). Such increases of anti-inflammatory markers within the circulation may provide positive metabolic changes through increased fat oxidation and glucose uptake (Pedersen, 2006; Pedersen & Febbraio, 2008; Walsh et al., 2011). We observed that both groups increased anti-inflammatory cytokines IL-6 and IL-1ra immediately post-exercise.
However, IL-6 remained elevated up to 60 min post-exercise and IL-1ra remained elevated up to 240 min post-exercise in the Caucasian group, but not the Indigenous group. This sustained elevation of IL-6 and IL-1ra following exercise in the Caucasian group may partially explain the differences observed between groups in the pre to post-exercise glucose response. Similarly, since cortisol is a stimulatory hormone that contributes to increased hepatic glucose production (Kindermann et al., 1982), and IL-6 stimulates peripheral glucose metabolism (muscle and adipose tissue) (Pedersen, 2006), the combined blunted cortisol response and increased IL-6 may explain the decreasing post-exercise plasma glucose concentration in the Indigenous group. In contrast, the Caucasian group showed a pre to post increase in cortisol and glucose concentration, which may explain the sustained elevation of IL-6 following exercise (Steensberg et al., 2001a). Moreover, the higher pre-exercise glucose concentration in the Indigenous group may have accounted for the blunted cortisol response to the exercise condition. While speculative, the known metabolic contribution of cortisol (stimulate hepatic glucose production) during exercise could infer that the metabolic requirements to the same relative intensity were different between groups and thus had a substantial impact on the post-exercise anti-inflammatory response. Given the small sample size and lack of previous literature, it is speculative to suggest there are ancestry dependent mechanisms responsible for these differences. However, the higher resting TNF-α and glucose concentrations within the Indigenous group may have contributed to the difference in post-exercise cortisol and anti-inflammatory responses between groups.

In conclusion, despite being matched for aerobic capacity and TB-FM%, there were differences in resting TNF-α and glucose concentrations between an Indigenous Australian and Caucasian group. This disparity at baseline may have contributed to different post-exercise anti-inflammatory responses (IL-6 and IL-1ra) between groups. Although the
Indigenous Australian group were self-identified, there is a high potential for genetic variability based on exposure to European influence for over 200 years. Accordingly, the genetic variability across multiple generations may influence the results obtained within this study and any future studies that are specific to comparing groups of differing ancestry. The similar post-exercise (0-240 min) glucose response between groups highlights the value of acute moderate-intensity exercise to be completed daily to assist with long-term improvements in glucose, irrespective of ancestry. Of note, the results of the current study suggest it to be even more pertinent for exercise to be encouraged within the Indigenous Australian population due to their elevated resting glucose levels at a younger age, when compared to the respective Caucasian group. Furthermore, exercise prescription within high-risk Indigenous populations should be designed to specifically focus on risk stratification, cultural sensitivity and thus ensure appropriate adaptations to exercise for chronic disease prevention. Whilst the outcomes of the current study are specific to Indigenous Australians and Caucasians, the findings highlight the need for additional research on exercise prescription involving acute differences within and between other Indigenous populations at high-risk of metabolic abnormalities.
CHAPTER FOUR

Differences in the acute inflammatory and glucose responses between rugby-specific small-sided games and cycle ergometry in middle-aged, inactive Caucasian men.

As accepted for publication

4.1: Abstract

**Aim:** This study compared the acute inflammatory and glucose responses within and between rugby specific SSG and stationary CYC in sedentary, middle-aged Caucasian men.

**Method:** Nine middle-aged, sedentary men who were free from disease participated in 2 x 40 min exercise conditions (CYC and SSG) following a randomised, cross-over design. HR and RPE were collected during each bout. Venous blood was collected at fasting, 0, 30, 60 and 240 min post-exercise for measurement of glucose, insulin, cortisol and inflammatory markers including TNF-α, IL-1β, IL-6, IL-1ra and CRP.

**Results:** No significant differences existed between conditions for HR and RPE (p>0.05). IL-6 was increased immediately post-exercise in both conditions (CYC 6.39 pg.mL⁻¹ and SSG 7.35 pg.mL⁻¹; p<0.05), but greater in SSG at 240 min post-exercise (6.95 pg.mL⁻¹) compared to CYC (4.18 pg.mL⁻¹; p<0.05). Glucose was lower in SSG than CYC at 30 (CYC 3.50 mmol.L⁻¹ and SSG 4.71 mmol.L⁻¹) and 240 min post-exercise (CYC 4.33 mmol.L⁻¹ and SSG 3.59 mmol.L⁻¹; p<0.05). IL-1ra, insulin and cortisol showed an exercise-induced increase (p<0.05), with no significant differences between conditions (p>0.05). Results for CRP, TNF-α and IL-1β showed no significant exercise-induced changes within or between conditions (p>0.05).

**Conclusion:** Both SSG and CYC conditions were sufficient to stimulate an acute anti-inflammatory response as indicated by the post-exercise elevation of IL-6, IL-1ra and cortisol. The novel findings are that an acute bout of SSG bout is capable of inducing and maintaining an elevated post-exercise IL-6 response and increased blood glucose disposal, compared with intensity- and duration-matched CYC condition.
4.2: Introduction

Chronic low-grade inflammation has been established as a predictor for the development of chronic diseases, such as T2DM and CVD (Pradhan et al., 2001). An inactive lifestyle is proposed to lead to the accumulation of adipose tissue and is accompanied by the infiltration of adipose derived pro-inflammatory proteins into the circulation (Pedersen et al., 2003). Conversely, increased physical activity has been reported as an effective preventative approach to reduce the inflammatory risks associated with these chronic metabolic and cardiovascular diseases (Kadoglou et al., 2007; You et al., 2013). Notably, the reduced inflammatory state from regular exercise is proposed to occur through the heightened anti-inflammatory environment induced by the acute bout (Ispirlidis et al., 2008; Ostrowski et al., 1999; Pedersen & Febbraio, 2008).

Acute exercise has been shown to stimulate glucose disposal and inhibit the release of pro-inflammatory cytokines (Helge et al., 2003). Indeed, the magnitude of the acute inflammatory and glucose regulatory response tends to be dictated by the cohort (trained and untrained), the muscle mass involved to complete the mechanical work, intensity and duration of the bout (Mendham et al., 2011; Pedersen et al., 2003; Steensberg et al., 2003). Typically following exercise, the active skeletal muscle increases both cellular and circulating levels of IL-6 (Steensberg et al., 2000). This acute increase in IL-6 is transient and produced independently to pro-inflammatory cytokines (TNF-α and IL-1β) (Starkie et al., 2003). Moreover, IL-6 has been shown to be responsible for a successive rise in anti-inflammatory cytokine IL-1ra (agonist to IL-1β), hepatic synthesis of CRP, the suppression of TNF-α, and the release of cortisol (Ostrowski et al., 1999; Starkie et al., 2003; Steensberg et al., 2003). In addition, the increased release of cortisol stimulates endogenous glucose production from the liver, while IL-6 has also been shown to increase basal and insulin-stimulated glucose uptake in skeletal
muscle via stimulation of the AMPK pathway and associated increase in GLUT4 translocation, while the increased release of cortisol stimulates endogenous glucose production from the liver (Carey et al., 2006; Pedersen & Febbraio, 2008). The increased concentration of both cortisol and IL-6 collectively regulate blood glucose concentration during acute exercise by maintaining equilibrium between glucose disposal and production.

Previous studies examining the acute exercise-induced inflammatory responses in sedentary populations have used gym-based methods of aerobic (i.e. cycling or running) and/or resistance (i.e. machine and free weights) exercises of differing intensities and durations (Mendham et al., 2011; Pedersen & Febbraio, 2008). However, group aerobic training sessions are reported to be more enjoyable than individualised training, which can potentially affect adherence and sustainability of an exercise training program (Irwin, Scorniaenchi, Kerr, Eisenmann, & Feltz, 2012). Recently, soccer specific SSG training has been reported to incorporate high-intensity intermittent sprints into an endurance-based event, which was highlighted as capable of inducing positive chronic adaptations (body composition, aerobic capacity, blood pressure, strength) either comparable to, or better than, traditional training modalities such as running (Krustrup et al., 2010a; Randers et al., 2012). To date, previous research on the acute post-exercise inflammatory response to SSG or intermittent sprint protocols has been specific to young athletic populations (Ispirlidis et al., 2008). A further understanding of these acute inflammatory and glucose responses in a sedentary, middle-aged population may be beneficial to justify the prescription of SSG for long-term inflammatory and glucose regulatory health benefits. Accordingly, the present study aimed to quantify and compare the acute inflammatory and glucose regulatory response within and between rugby-specific SSG and CYC conditions in sedentary, middle-aged Caucasian men. It was
hypothesised that when matched for intensity and duration between the conditions a similar inflammatory and glucose regulatory response will be evident.

4.3: Methods

4.3.1: Participant recruitment

The study population comprised of 9 sedentary, middle-aged men (48.8 ±1.7y) who were not clinically diagnosed with any pre-existing cardiovascular or metabolic disorders. The sedentary criteria ensured those completing no more than one regular exercise session per week (>20min) within the preceding 6 months. Those excluded were those with immunological irregularities, smokers (<2 y cessation); those suffering from recurrent or recent influenza illness (including flu shot recipients); those on cholesterol lowering, anti-inflammatory, or any other medication/condition reported to affect the inflammatory response (i.e. rheumatoid arthritis or periodontal disease). Prior to participant recruitment the study was approved by the Research in Human Ethics Committee of the University. All participants provided verbal and written consent prior to the commencement of testing procedures.

4.3.2: Overview

In a randomised cross-over design participants completed CYC and SSG conditions, each separated by 21 d to allow adequate recovery from an unaccustomed exercise session. Testing procedures commenced between standardised times (6-8 am), following an overnight fast (10-12 h). Based on written and verbal confirmation, physical activity and diet were standardised and recorded 24 h prior to testing and replicated for the remaining condition. During each condition and 240 min after all testing sessions participants remained fasted and consumed water *ab libitum* (~500 mL).
4.3.3: Baseline testing

At pre-intervention testing, stature (stadiometer: Custom CSU, Bathurst, Australia), body mass (HW 150 K; A&D, Bradford, MA, USA), waist and hip girths (EC P3 steel tape Sydney, Australia) were obtained from all participants. Manual blood pressure was obtained with an aneroid sphygmomanometer and cuff (Welch-Allyn, Arden, North Carolina, USA) expressed as the mean of three measurements after being seated for 5-min. A supine whole body DXA scan (XR800, Norland, Cooper Surgical Company, USA) was conducted with scanning resolution set at 6.5 x13.0 mm, and scanning speed was set at 130 mm·s⁻¹. Scans were analysed (Illuminatus DXA, ver.4.2.0, USA) for TB-FFM and TB-FM (Kim et al., 2002).

Aerobic capacity was determined from measurement of VO₂ (Parvo Medics, True2400, East Sandy, Utah, USA) during a sub-maximal GXT, which was used in preference to maximal testing to minimise associated risks in sedentary, middle-aged men (Wallman & Campbell, 2007). Prior to each session, the ventilometer was calibrated using a three-litre syringe, while gas analysers were calibrated for fractional gas concentration with a gravimetric gas mixture of known concentrations (CO₂, 4.1 ±0.08%; O₂, 15.7 ±0.2%), in accordance with the manufacturer’s instructions. The GXT was performed on an electronically braked cycle ergometer (LODE Excalibur Sport, LODE BV, Groningen, The Netherlands), which started at 25 W and increased by 25 W every minute. HR (Vantage NV, Polar, Finland) was continuously monitored, and participants exercised until attainment of 80% age-predicted (220-age) HR\textsubscript{max}. 

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4.3.4: Small-sided games condition

The SSG condition involved modified football (non-contact rugby league) as this is the most popular football code in this geographical region (Kennett et al., 2012). Participants completed 40 min of 6 v 6 on a reduced-size pitch (width: 40 m; length: 60 m) to induce a mean target HR zone ~80–85% HR_max. The session comprised of 4 x 10 min bouts, interspersed by 2 min passive recovery. Speed was recorded every second using a 1Hz GPS device (SPI-Pro, 1 Hz, GPSports, Canberra, ACT, Australia). The GPS unit was worn in a customised harness between the scapulae to quantify distance and mean speed (m·min⁻¹) of movement patterns during the session (Coutts & Duffield, 2010). At the end of each 10 min period, HR and RPE (Borg’s 6-20 scale) were recorded, as well as a session-RPE 30min post-exercise (Foster et al., 2001).

4.3.5: Cycle ergometry condition

The CYC condition was conducted on a Monark stationary cycle ergometer (Monark 828E, Monark Exercise AB, Varburg, Sweden) and comprised of 4x10 min bouts, interspersed by 2 min passive recovery. During the session, cadence was maintained at 60-65 rpm and individual resistance adjusted to maintain target HR zones. Given the different exercise modes conditions it is recognised the inherent difficulties of matching external load or metabolic cost. However, despite this limitation, in an attempt to best match the intensity between conditions the respective conditions were designed to elicit similar internal loads. At the end of each 10 min interval HR and RPE were recorded, including session-RPE 30 min following exercise. The intensity and duration of the CYC condition was design to match the SSG condition and approximate mean target HR zone of 80–85% HR_max.
4.5.6: Venous blood sampling and analyses

Serum or plasma was collected following centrifugation at 3500 rpm for 15 min at 4°C. Aliquots were frozen at -80°C and -20°C for EDTA and SST, respectively. Whole blood in EDTA tubes was refrigerated (4°C) for a maximum of 6 h until analysis for total leukocyte count and HbA1c. Fluoride oxalate tubes were refrigerated (4°C) for a maximum of 30 min until analysis of glucose and lactate. Blood was collected during baseline testing for analysis of fasting total cholesterol, high density lipoprotein, triglycerides (Enzymatic method and biochromatic endpoint technique; Dimension Xpand Plus, Siemens Healthcare Diagnostics, Sydney, Australia), total leukocyte count (Cell Counter: Cell-Dyn 3200, Abbott Laboratories, IL, USA) and HbA1c (Liquid Chromatography: Bio-Rad Variant, Sydney, Australia). During each condition, 20 mL was collected at each time point for analysis of glucose, lactate (ABL825 Flex Analyzer, Radiometer Medical ApS, Bronshoj, Denmark), insulin, cortisol (Chemiluminescent Immunometric Assay: Immulite 2000, Siemens Healthcare Diagnostics, Los Angeles, CA, USA) and CRP (Particle enhanced turbidimetric immunoassay Dimension Xpand Plus, Siemens Healthcare Diagnostics, Sydney, Australia). Analysis of biochemistry variables glucose, insulin, lactate, cortisol and CRP showed intra and inter-assay coefficients of variation of 4.0-7.4%. IL-6, IL-1β, IL-1ra and TNF-α were measured at each time point (ELISA Immunoassay: R & D Systems, Minneapolis, MA, USA), with intra and inter-assay coefficients of variation of 4.3-5.6%. HOMA-IR was calculated using the formula (fasting insulin x fasting glucose)/22.5 (Matthews et al., 1985).

4.3.7: Statistical analyses

All data are reported as mean ±SEM. Within and between condition and time-point differences were assessed using two-way repeated measures ANOVA (condition x time). When significant interactions were observed, Tukey’s pairwise comparisons were employed.
to assess the source of significance, which was set at p<0.05. All statistical analyses were performed using PASW™ for MS-Windows v17.0 (Statistical Package for the Social Sciences, Chicago, IL, USA).

4.4: Results

All participant characteristics (anthropometry, body composition, VO₂ and fasting blood chemistry) are presented in Table 4.1. Glucose, insulin and CRP concentration were collected prior to each exercise condition, thus, baseline values presented in Table 4.1 are the mean between the two respective pre-exercise time-points.
Table 4.1
Participant baseline characteristics (n=9). Data reported as mean ± SEM

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>48.8</td>
<td>± 1.7</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.77</td>
<td>± 0.02</td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>101.6</td>
<td>± 3.3</td>
</tr>
<tr>
<td>Sub-maximal VO$_2$ (mL kg$^{-1}$ min$^{-1}$)</td>
<td>30.8</td>
<td>± 1.3</td>
</tr>
<tr>
<td>Body mass index (kg m$^2$)</td>
<td>32.2</td>
<td>± 0.8</td>
</tr>
<tr>
<td>Waist to Hip Ratio</td>
<td>0.98</td>
<td>± 0.02</td>
</tr>
<tr>
<td>Total Body-Fat Mass (%)</td>
<td>31.4</td>
<td>± 1.6</td>
</tr>
<tr>
<td>Total Body-Fat Mass (kg)</td>
<td>31.8</td>
<td>± 2.7</td>
</tr>
<tr>
<td>Total Body-Fat Free Mass (kg)</td>
<td>64.3</td>
<td>± 1.2</td>
</tr>
<tr>
<td>Systolic Bp (mmHg)</td>
<td>131</td>
<td>± 4</td>
</tr>
<tr>
<td>Diastolic Bp (mmHg)</td>
<td>82</td>
<td>± 2</td>
</tr>
<tr>
<td>HbA1c (%A1c)</td>
<td>5.7</td>
<td>± 0.1</td>
</tr>
<tr>
<td>Glucose (mmol L$^{-1}$)</td>
<td>4.6</td>
<td>± 0.3</td>
</tr>
<tr>
<td>Insulin (µU mL$^{-1}$)</td>
<td>11.5</td>
<td>± 2.9</td>
</tr>
<tr>
<td>Insulin resistance (HOMA-IR)</td>
<td>2.4</td>
<td>± 0.6</td>
</tr>
<tr>
<td>Total Cholesterol (mmol L$^{-1}$)</td>
<td>5.8</td>
<td>± 0.4</td>
</tr>
<tr>
<td>Low Density Lipoprotein (mmol L$^{-1}$)</td>
<td>3.4</td>
<td>± 0.4</td>
</tr>
<tr>
<td>High Density Lipoprotein (mmol L$^{-1}$)</td>
<td>1.3</td>
<td>± 0.1</td>
</tr>
<tr>
<td>Triglycerides (mmol L$^{-1}$)</td>
<td>2.2</td>
<td>± 0.4</td>
</tr>
<tr>
<td>Cholesterol hazard ratio (Total : HDL)</td>
<td>4.6</td>
<td>± 0.4</td>
</tr>
<tr>
<td>Total Leukocyte Count (10$^9$ L$^{-1}$)</td>
<td>6.1</td>
<td>± 0.4</td>
</tr>
<tr>
<td>C-Reactive Protein (mg L$^{-1}$)</td>
<td>2.5</td>
<td>± 0.5</td>
</tr>
</tbody>
</table>
4.4.1: Small-sided games and cycle ergometry demands

Total distance covered during the SSG was 3173 ±104 m, at a speed of 79 ±3 m·min⁻¹, with 146 ±91 m of high-speed running above 14 km·h⁻¹. Mean resistance for the CYC condition was 1.9 ±0.2 kp. No significant differences were evident between conditions for %HR_max (SSG 85.6 ±1.9% HR_max; CYC 83.8 ±1.2% HR_max; p=0.22) and session-RPE (SSG, 13.2 ±0.4 AU; CYC, 13.6 ±0.4 AU; p=0.40). Blood lactate peaked immediately post-exercise at 2.27 ±0.40 and 2.08 ±0.46 mmol·L⁻¹ for CYC and SSG, respectively (p>0.05).

4.4.2: Acute inflammatory responses

The acute IL-6, IL-1ra, TNF-α, and IL-1β response of SSG and CYC conditions are shown in Figure 4.1. The acute IL-6 response shows a significant increase immediately post-exercise within both conditions (p<0.05). Significant differences were only evident between conditions at 240 min post-exercise (p=0.04) with SSG remaining elevated above pre (p=0.005), though not in CYC (p=0.15). IL-1ra was significantly increased immediately post-exercise (p<0.05) and remained elevated above pre values at 240 min in both SSG and CYC (p<0.05), without significant differences between conditions (p>0.05). Results for CRP, TNF-α and IL-1β showed no significant changes within or between conditions (p>0.05).
Figure 4.1
Mean ± SEM response of IL-6, IL-1ra TNF-α and IL-1β within and between the respective conditions. Significant difference from baseline within the cycling condition \( a \) \( P<0.05 \); Significant differences from baseline within the small-sided games condition \( b \) \( P<0.05 \); Significant difference between groups \( c \) \( P<0.05 \).
4.4.3: Acute cortisol, glucose, insulin and HOMA-IR responses

The acute cortisol, glucose, insulin and HOMA-IR responses of SSG and CYC conditions are presented in Figure 4.2. Cortisol was significantly increased in both conditions immediate post-exercise (p<0.05), though decreased at 60 min (p<0.05) and remained below pre-values at 240 min post-exercise (p<0.05), without differences between conditions (p>0.05). In both conditions glucose increased immediately post-exercise (p<0.05) followed by a significant decline at 30 min (p<0.05). Glucose concentrations for SSG were significantly lower than CYC at 30 min (p=0.02) and 240 min post-exercise (p=0.03). Insulin concentrations showed a significant decline below pre-values at 240 min post-exercise in SSG (p=0.02) and CYC (p=0.01), with no significant difference between conditions (p=0.61). HOMA-IR increased immediately post-exercise in SSG (p=0.028). Differences between conditions were evident in the change from pre to 0 min (p=0.038) and 0 min to 30 min post-exercise (p=0.012).
Figure 4.2
Mean ± SEM response of cortisol, glucose, insulin and HOMA-IR within and between the respective conditions. Significant differences from baseline within the cycling condition a $P<0.05$; Significant differences from baseline within the small-sided games condition b $P<0.05$; Significant difference between groups c $P<0.05$. 
The inactive, middle-aged men recruited for the present study can be classified as obese with elevated CRP, high cholesterol hazard ratio and low aerobic capacity (Alberti et al., 2006; Pradhan et al., 2001). These characteristics place the participants at ‘high risk’ of developing metabolic and cardiovascular diseases (Alberti et al., 2006). Both SSG and CYC conditions elicited an acute anti-inflammatory response as evidenced by the post-exercise elevation of IL-6 and IL-1ra, while no exercise-induced changes were evident in pro-inflammatory markers IL-1β, TNF-α and CRP. The novel findings were that an acute bout of SSG is capable of inducing and maintaining an elevated post-exercise IL-6 response and increased blood glucose disposal when compared to intensity- and duration-matched CYC condition. Regardless, both SSG and CYC conditions stimulated glucose disposal and provided a post-exercise anti-inflammatory milieu several hours after the exercise bout.

During muscle contraction IL-6 is released into the circulation from the active myocytes, which initiates an anti-inflammatory environment by stimulating the release of IL-1ra and cortisol into the circulation (Pedersen et al., 2003; Steensberg et al., 2003; Steensberg et al., 2000). The present findings showed that despite both conditions showing a similar response in IL-6 immediately post-exercise, SSG elevated IL-6 above CYC at 240 min. It is consistently reported that the IL-6 response to acute exercise varies according to whether the cohort is trained or untrained, and indicative of intensity, duration and the muscle mass required to complete the mechanical work (Mendham et al., 2011; Pedersen et al., 2003; Steensberg et al., 2003). Furthermore, given the attempt to match intensity (via internal load i.e. cardiovascular strain) and duration between conditions, the recruitment of greater muscle mass (i.e. both upper and lower body are involved) in the SSGs may explain sustained elevation in plasma IL-6. Irrespective, these results show an increase of IL-6 in response to
acute CYC and SSG conditions; whether the elevated response from SSG leads to chronic adaptations is yet to be elucidated.

Consistent with previous research, IL-1ra peaked at 60 min post-exercise and remained elevated for up to 240 min in both conditions (Mendham, Coutts, & Duffield, 2012; Ostrowski et al., 1999). These results suggest the release of IL-6 and subsequent secretion of IL-1ra represents as an exercise-induced anti-inflammatory environment within both SSG and CYC conditions (Fischer, 2006; Steensberg et al., 2003). Differences between conditions for IL-6 occurred at 240min post-exercise; hence, the effect on IL-1ra is unknown because it is likely that any differences may occur after the 240 min time-point. Regardless, both SSG and CYC exercise stimulates positive acute anti-inflammatory responses, with a greater response in SSG. Future studies should investigate if the different acute anti-inflammatory response between SSG and CYC impacts on chronic inflammatory training adaptations for the prevention and treatment of T2DM and CVD (You et al., 2013).

The increased concentrations of IL-6 and IL-1ra create an anti-inflammatory environment which has been shown to inhibit the release of pro-inflammatory cytokines, such as TNF-α and IL-1β (Schindler et al., 1990; Starkie et al., 2003). The present study showed no post-exercise change in plasma concentrations of pro-inflammatory (TNF-α and IL-1β) cytokines. These results are consistent with different models of moderate-intensity aerobic conditions (i.e. running, intermittent running, SSG and cycling) within sedentary populations (Harris, Padilla, Hanlon, Rink, & Wallace, 2008; Mendham et al., 2012). The non-significant changes reported in the current and previous studies may be due to the sedentary characteristics of the participants and/or the prescribed exercise intensity; it is likely that more strenuous and longer duration exercise is required changes resulting in immunological strain and elevated
post-exercise IL-1β and TNF-α (Ostrowski et al., 1999). These strenuous and long duration training methods are generally not prescribed with sedentary middle-aged cohorts hence the lack of literature describing changes in pro-inflammatory markers in response to acute exercise.

Prolonged and intermittent exercise can also lead to the release of CRP within 24–48 h following exercise - with the magnitude of increase dependant on the hepatic synthesis of IL-6 and/or muscle damage (Cunniffe et al., 2010; Ispirlidis et al., 2008). The present study showed no exercise-induced response to SSG or CYC in plasma CRP. A limitation is that CRP was only measured up to 240 min post-exercise and may explain the negligible response observed within CYC and SSG conditions. Previous research has shown an increase in CRP 24 h post-exercise in accordance with an increase in muscle damage markers (CK, myoglobin) from high-intensity resistance exercise compared to intensity and duration-matched cycling (Mendham et al., 2011). As such, future research should assess CRP within the 24–48 h period post SSG in accordance within muscle damage, when compared to CYC. This may provide a mechanism within sedentary populations to determine the influence of concentric and eccentric modalities as indicative of respective CYC and SSG modes and resultant influence on post-exercise inflammatory cascades.

Cortisol is known to have potent anti-inflammatory effects, which can also be augmented by the increase in IL-6 and subsequent down regulation of pro-inflammatory cytokines (TNF-α and IL-1β) by immune cells (Ostrowski et al., 1999; Pedersen et al., 2003; Steensberg et al., 2003). The cortisol response to both aerobic and resistance exercise is dependent on intensity and duration, more so than the exercise mode (Kindermann et al., 1982; Mendham et al., 2011). Accordingly, the current study showed a similar cortisol response between SSG and
Chapter 4: Study 2

CYC conditions matched for duration and intensity. Specifically, cortisol functions as an energy sensor to augment hepatic glucose release and provide sufficient fuel for metabolic demands (Steensberg et al., 2003). The present study shows SSG resulted in a reduced post-exercise glucose concentration when compared to CYC, despite Rugby-specific small-sided games as well as continuous cycle exercise showing similar reductions in insulin at 240min post-exercise. As such, two potential reasons for the difference in post-exercise glucose concentration may relate to the respective differences between conditions in HOMA-IR and IL-6 post-exercise response. Firstly, HOMA-IR increased immediately post-exercise in the SSG condition, which indicates a necessary release of insulin to lower blood glucose concentration within the post-exercise recovery period (Goodyear & Kahn, 1998). Secondly, IL-6 stimulates the AMP-Kinase signalling pathway and associated glucose disposal through GLUT4 translocation (Helge et al., 2003). As such the sustained elevation of IL-6 in the SSG conditions may have contributed to increased blood glucose disposal when compared to CYC (Helge et al., 2003; Pedersen & Febbraio, 2008). However, further research is required specific to skeletal muscle energy signalling pathways and associated glucose regulation induced by the respective conditions. Taken collectively, the increase HOMA-IR immediately post-exercise alongside an amplified IL-6 response are two potential mechanisms simultaneously causing an increase in peripheral glucose metabolism and thus a lower plasma glucose concentration in SSG, compared an intensity- and duration-matched CYC condition.

4.5.1 Conclusion

Both conditions stimulated glucose disposal and provided a post-exercise anti-inflammatory milieu several hours after the exercise bout. The novel findings were that an acute bout of SSG is capable of maintaining an elevated post-exercise IL-6 response and increased blood
glucose disposal when compared to CYC. Given these respective differences between conditions and the lack of inflammatory based research within SSG training for sedentary populations, it is suggested future studies examine the chronic adaptations of SSG training and its role for long-term health benefits.
CHAPTER FIVE

Rugby-specific small-sided games training is an effective alternative to improve the chronic inflammatory state compared to continuous cycle ergometry in middle-aged, inactive Caucasian men

As accepted for publication

5.1: Abstract

Aim: To assess changes in pro- and anti-inflammatory cytokines, aerobic capacity and body composition following 8-weeks of either SSG or CYC training compared to a sedentary control (CON) condition.

Methods: Thirty-three middle-aged (age 48.6 ±2.0 y), inactive men were randomised into a CYC (n=11), SSG (n=11), or CON (n=11) group. Participants in exercise groups trained 3d wk⁻¹ for 8 weeks, while control participants maintained normal activity and dietary patterns. Groups were matched on exercise duration with CYC performed continuously and SSG separated into four quarters interspersed with 2-min passive recovery periods. Both training programs were designed to elicit similar heart rates (~80-85% HRmax) and Rating of Perceived Exertion (RPE). Pre- and post-intervention testing included a DXA and fasting venous blood. Venous blood measures for pro-inflammatory markers included CRP, IL-6, IL-1β, TNF-α, and leptin; anti-inflammatory markers included IL-10, IL-1ra, and adiponectin.

Results: Both CYC and SSG decreased TB-FM (p<0.05), and CRP (SSG, -15.0±4.1%, p=0.008; CYC, -14.0±5.4%, p=0.02). Only SSG increased TB-FFM (2.0±0.7%, p=0.017) and decreased concentration of plasma IL-6 (-30.6±6.1%; p=0.002) and leptin (-20.6±7.0%; p=0.01).

Conclusion: Cycling and SSG training were both effective at improving CRP, VO₂ and TB-FM. However, differences between conditions show SSG to be a more effective training approach in reducing IL-6 and leptin and increasing muscle mass within sedentary, middle-aged men.
5.2: Introduction

Physical inactivity and obesity are risk-factors that contribute to a heightened systemic inflammatory state and the overall development of T2DM and CVD (Pradhan et al., 2001; You et al., 2013). A sub-clinical inflammatory state is evident from elevated plasma concentrations of pro-inflammatory markers such as leptin, IL-6, IL-1β, TNF-α and CRP (Popa et al., 2007; Pradhan et al., 2001). In response, the anti-inflammatory marker IL-1ra is elevated in an attempt to suppress IL-1β and retain homeostasis of the innate immune system (Chernoff et al., 1995; Petersen & Pedersen, 2005). Conversely, anti-inflammatory markers such as adiponectin and IL-10 are known to improve endothelial function, insulin sensitivity and the inhibition of pro-inflammatory mediators (Arita et al., 1999; Bouassida et al., 2010). However, these anti-inflammatory markers are suppressed in obese and insulin resistance populations and in coordination with increased pro-inflammatory markers, results in a sub-clinical inflammatory state and the development of metabolic and cardiovascular abnormalities (Arita et al., 1999; Bouassida et al., 2010).

Long-term physical activity can reduce the chronic inflammatory state and protect against the development of chronic disease (Petersen & Pedersen, 2005). Participants who engage in high levels of physical activity reportedly have lower basal concentrations of CRP (29%), IL-6 (32%) and TNF-α (20%) when compared to sedentary participants (Panagiotakos et al., 2005). Furthermore, elevated concentrations of IL-6 (>1.80 pg·mL⁻¹) and TNF-α (>3.20 pg·mL⁻¹) have been shown to be associated with low muscle mass and increased fat-mass (Visser et al., 2002). Given that the chronic inflammatory state is reportedly mediated by the ratio of adiposity to muscle mass, the extent to which the exercise mode alters these variables of body composition may have a bearing on altering the inflammatory state (Carson et al., 2012; Fried et al., 1998; Visser et al., 2002). Ongoing engagement in either aerobic (i.e.
walking, running or cycling) and/or resistance training is proposed to result in reductions in pro-inflammatory (i.e. CRP, IL-6, TNF-α, leptin) and an increased anti-inflammatory (i.e. IL-10, adiponectin) markers, although these current findings remain equivocal (Bouassida et al., 2010; Donges et al., 2010; You et al., 2013). Possible reasons for these discrepancies in the inflammatory responses are the divergent adaptations in body composition (i.e. fat mass and muscle mass) between the exercise modes (Carson et al., 2012; Fried et al., 1998; Visser et al., 2002).

Small-sided games are a popular health promotion initiative within sedentary populations (Krustrup et al., 2010d; Krustrup et al., 2009). For those impartial towards individual aerobic exercise sessions such as stationary cycling, SSG is a group training approach that delivers repeated bouts of high-intensity efforts over prolonged durations (Randers et al., 2010b). Training studies which have assessed soccer-specific SSG in sedentary populations have shown positive adaptations in aerobic capacity and body composition (i.e. reduced fat-mass and increased muscle mass) (Krustrup et al., 2010b), which may have a potential influence on the chronic inflammatory state (Bouassida et al., 2010; Gleeson et al., 2011). However, to our knowledge no studies have assessed the pro- and anti-inflammatory response to rugby-specific SSG training and/or in comparison to traditional training modes such as stationary cycling within a sedentary cohort. The aim of the present study was to assess the training-induced changes in pro- and anti-inflammatory cytokines, aerobic capacity and body composition in response to 8-weeks of SSG, stationary CYC or CON conditions. It was hypothesised that both SSG and CYC training will improve the anti- and pro-inflammatory state, body composition and aerobic capacity.
5.3: Methods

5.3.1: Participant recruitment

Thirty-three men (Table 6.1; age 48.6 ±2.1 y; stature 176.7 ±1.8 cm; mass 89.8 ±3.7 kg) were recruited and randomly assigned to a stationary cycling (CYC, n=11), a SSG (n=11), or control (CON, n=11) group (Figure 6.1). Participant allocation to conditions was conducted through block randomization (Microsoft Office, excel 2010) in groups of 3 by a research assistant. Participant recruitment ensured a non-smoking population representative of a sedentary lifestyle (no regular pattern of planned or incidental activity >60 min wk⁻¹). Participant exclusion criteria extended to those on or having received any medications, flu injections or vitamin supplementations and those clinically diagnosed with any pre-existing CVD, metabolic or inflammatory related disorders. Participants with orthopaedic limitations were advised against involvement due to the musculoskeletal demands of the respective exercise protocols. Prior to pre-intervention testing procedures clearance was obtained from the University Ethics in Human Research Committee and participants attended an information and familiarisation session where verbal and written consent for all testing and training procedures was obtained.
Table 5.1

Participant characteristics, body composition and graded-exercise test to 80% HR_{max} pre and post 8-weeks of small-sided games (n=10), cycle ergometry (n=11) or control (n=11) condition. Data reported as mean ± SEM.

<table>
<thead>
<tr>
<th></th>
<th>Small-Sided Games</th>
<th>Cycle Ergometry</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>Age (y)</td>
<td>46.8 ± 2.1</td>
<td>-</td>
<td>49.5 ± 2.0</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.76 ± 0.02</td>
<td>-</td>
<td>1.76 ± 0.01</td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td>86.3 ± 4.3</td>
<td>86.1 ± 4.2</td>
<td>90.3 ± 3.7</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>27.6 ± 0.9</td>
<td>27.6 ± 0.9</td>
<td>29.1 ± 1.1</td>
</tr>
<tr>
<td>WHR</td>
<td>0.95 ± 0.02</td>
<td>0.94± 0.01</td>
<td>0.95± 0.02</td>
</tr>
</tbody>
</table>

**DXA analysis**

<table>
<thead>
<tr>
<th></th>
<th>Small-Sided Games</th>
<th>Cycle Ergometry</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>TB-FM (kg)</td>
<td>23.8 ± 1.9</td>
<td>23.1 ± 1.9 ab</td>
<td>26.7 ± 2.5</td>
</tr>
<tr>
<td>TB-FM (%)</td>
<td>27.2 ± 0.9</td>
<td>26.2 ± 1.0 ab</td>
<td>28.9 ± 1.9</td>
</tr>
<tr>
<td>TB-FFM (kg)</td>
<td>59.3 ± 2.5</td>
<td>60.4 ± 2.5 ab</td>
<td>60.3 ± 1.9</td>
</tr>
</tbody>
</table>

BMI, Body mass index; WHR, waist to hip ratio; DXA, Dual-energy x-ray absorptiometry; TB-FM, Total body-fat mass; TB-FFM, Total body-fat free mass; GXT, graded exercise test; VO\(_2\), Oxygen consumption.

a = Significant within group change (p<0.05); b = Significant change compared to control group (p<0.05)
5.3.2: Overview

Participants attended a pre- and post-intervention testing session. These testing sessions comprised of a pre-screening health questionnaire, anthropometry, a DXA scan, and a fasting (10-12 h) blood sample. The training interventions consisted of 8 weeks of rugby-specific SSG or CYC and participants were required to attend 90% of all training sessions for inclusion in the post-training testing. Each training session consisted of a 5 min warm-up, which included a slow jog (paced at 8.5 km·h⁻¹) for the SSG group and a slow cycle (2 kp at 60 rpm) for the cycling group. Given the different exercise modes used during respective training programs, it is recognised the inherent difficulties of matching external training load or metabolic cost. However, despite this limitation, in an attempt to match training load between conditions the respective training programs were designed to elicit similar heart rates and session RPE values. Heart rate (Vantage NV, Polar, Kempele, Finland) was recorded at 5-min intervals and reported as mean percent of age-predicted (220 – age) % HRmax and session-RPE (Borg 6-20 scale) at the conclusion of each training session (Foster et al., 2001).

5.3.3: Nutrition and physical activity standardisation

Prior to pre and post training interventions participants refrained from any physical activity for 72 h, and the consumption of alcohol and caffeine for 24 h. Participants documented dietary and physical activity patterns 24 h prior to pre intervention testing. This diary was photocopied and issued to the participants to ensure diet was replicated for the 24-h period prior to post-intervention testing. Participants were informed of the importance in maintaining their normal dietary and nutritional patterns throughout the 8-week training period. Participants were required to maintain food and beverage type and timing of consumption, including cooking preparation and portion size. Physical activity was
standardised to ensure all participants did not engage in any additional planned or incidental physical activity, nor reduce any incidental activity during the 8-week intervention.

5.3.4: Stationary cycle ergometry condition

The training program involved participants performing continuous cycle ergometry (Monark 828E, Monark Exercise AB, Varburg, Sweden) 3 d\textsuperscript{ wk}\textsuperscript{-1} (details of external training load and progression are presented in Table 5.2). To quantify external training load, kp, rpm and total distance (km) were recorded at 5-min intervals during each session, with training load progression manipulated through alternate increases in session duration and resistance (kp), respectively.

5.3.5: Small-sided games condition

The SSG group involved 3 d\textsuperscript{ wk}\textsuperscript{-1} of modified rugby league (most popular football code in this geographical region). Each modified rugby session was played under Touch Football rules (Kennett et al., 2012). The rules allowed each team six ‘plays’ whilst in possession of the ball; each play required players to pass the ball backwards to an ‘on side’ team member with the aim to score at opposing ends of the field. Defending players were required to touch their opponent with one hand. Following a successful touch, game play would restart with a ‘play the ball’, at this time requiring the line of defending players to be 5 m from the position of each ‘play the ball’ (Kennett et al., 2012). Each training session comprised of 4 x 10 min quarters, interspersed by 2 min passive recovery periods (see Table 5.2 for details of external training load and progression). To quantify external training load, a GPS device (SPI-Pro, 1 Hz, GPSports, Canberra, ACT, Australia) was worn in a customised harness between the scapulae to quantify total distance (m), mean speed (m\textsuperscript{ min}^{-1}) and peak speed (km\textsuperscript{ h}^{-1}) of each training session. During the 8 weeks, training load progressively increased via manipulation of
session duration and field size, including consistent game rules, verbal feedback and player numbers at 5 v 5 or 6 v 6 (depending on participant availability).

5.3.6: Control condition
The CON group completed all pre and post-intervention testing sessions and was required to continue their sedentary life and habitual dietary and nutritional patterns for the 8-week intervention period. Participants were provided with a dietary and a physical activity diary that documented any dietary or physical activity changes. Participants received instruction expressing the importance of maintaining these patterns. The chief investigator reviewed the diaries prior to post-intervention testing to ensure individual conformity to the control condition.

5.3.6: Anthropometry and DXA
All testing procedures were conducted at a standardised time for each participant between 0600 and 0900 h. Anthropometric measures included stature (Stadiometer, CSU, Bathurst, Australia), body mass on calibrated scales (HW 150 K; A&D, Bradford, MA, USA) and waist and hip girths (EC P3 steel tape Sydney, Australia) for calculation of BMI and WHR (Marfell-Jones et al., 2012). A supine whole body DXA scan (XR800, Norland, Cooper Surgical Company, USA) was conducted with scanning resolution set at 6.5 x 13.0 mm, and scanning speed was set at 130 mm s⁻¹. Whole body scans were analysed (Illuminatus DXA, ver. 4.2.0, USA) for TB-FM and TB-FFM.

5.3.7: Venous blood collection and analyses
A fasting venous blood sample was collected for analysis of TNF-α, IL-6, IL-1β, IL-1ra, IL-10, leptin, adiponectin (Immunoassay ELISA: Quantikine, R & D Systems, Minneapolis,
MN, USA) and CRP (Particle enhanced turbidimetric immunoassay: Dimension Xpand Plus, Siemens Healthcare Diagnostics, Sydney, Australia). Intra and inter-assay coefficients of variation were between 2.2-4.9%. Following the clotting of the sample (SST) or immediately following collection (EDTA) samples were centrifuged at 3500 rpm for 15 min at 4°C. Aliquots were frozen immediately at -80°C until further analysis.

5.3.8: Statistical analyses

All data are reported as mean ±SEM. Non-normally distributed variables CRP, IL-10, IL-6, TNF-α, IL-1β, leptin and adiponectin were log transformed prior to all analysis. A general linear model two-way repeated measures ANOVA was used to compare conditions over time. Post hoc analysis to determine the location of differences when a significant main effect or interactions were detected was performed using a paired t-test with Tukey’s adjustment within conditions over time and between conditions at each time point. Significance was accepted at p<0.05. All statistical analyses were performed using PASW™ for MS-Windows Version 20.0 (Statistical Package for the Social Sciences, Chicago, IL, USA).

5.4: Results

5.5.1: Training load and compliance

Final numbers for the completion of this study were CON (n=11), CYC (n=11) and SSG (n=10). One participant could not complete the SSG training due to a knee injury sustained during training (week 3). Compliance to both training interventions was also not significantly different between groups (91 ±2% and 95 ±2% for SSG and CYC, respectively; p=0.127). Mean weekly training load variables and progression for SSG and CYC conditions are shown in Table 5.2. Mean % HR_{max} (SSG, 85.2 ±0.3; CYC, 84.6 ±0.4%; p=0.112) and session-RPE
(SSG, 12.4 ±0.1; CYC, 12.5 ±0.1 AU; p=0.641) were not significantly different between conditions.

Table 5.2

Training load and progression of 8-weeks training within small-sided games (n=10) and cycle ergometry (n=11). Data reported as mean ±SEM.

<table>
<thead>
<tr>
<th>Week</th>
<th>Duration (min)</th>
<th>Total Distance (m)</th>
<th>Mean speed (m/min(^1))</th>
<th>Peak Speed (km/h(^1))</th>
<th>Field Size</th>
<th>Total Distance (km)</th>
<th>kp</th>
<th>RPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40</td>
<td>2563 ± 53</td>
<td>71.5 ± 1.6</td>
<td>20.0 ± 0.6</td>
<td>25m; 40m</td>
<td>17.2 ± 0.3</td>
<td>1.5 ± 0.1</td>
<td>72.1 ± 1.3</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>2642 ± 74</td>
<td>73.4 ± 2.1</td>
<td>18.6 ± 0.5</td>
<td>25m; 40m</td>
<td>17.7 ± 0.4</td>
<td>1.7 ± 0.1</td>
<td>73.7 ± 1.5</td>
</tr>
<tr>
<td>3</td>
<td>45</td>
<td>2856 ± 59</td>
<td>71.3 ± 1.5</td>
<td>20.0 ± 0.6</td>
<td>25m; 40m</td>
<td>20.5 ± 0.3</td>
<td>1.9 ± 0.1</td>
<td>76.0 ± 1.2</td>
</tr>
<tr>
<td>4</td>
<td>45</td>
<td>3014 ± 63</td>
<td>75.1 ± 1.6</td>
<td>21.2 ± 0.6</td>
<td>35m; 50m</td>
<td>19.9 ± 0.3</td>
<td>2.2 ± 0.1</td>
<td>73.7 ± 1.0</td>
</tr>
<tr>
<td>5</td>
<td>45</td>
<td>3117 ± 62</td>
<td>77.8 ± 1.5</td>
<td>20.2 ± 0.7</td>
<td>35m; 50m</td>
<td>19.5 ± 0.3</td>
<td>2.2 ± 0.1</td>
<td>72.3 ± 1.2</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>3541 ± 70</td>
<td>78.8 ± 1.6</td>
<td>20.9 ± 0.6</td>
<td>35m; 50m</td>
<td>21.0 ± 0.3</td>
<td>2.2 ± 0.1</td>
<td>70.1 ± 1.0</td>
</tr>
<tr>
<td>7</td>
<td>50</td>
<td>3673 ± 59</td>
<td>82.0 ± 1.2</td>
<td>20.3 ± 0.7</td>
<td>40m; 60m</td>
<td>20.8 ± 0.3</td>
<td>2.4 ± 0.1</td>
<td>69.3 ± 0.9</td>
</tr>
<tr>
<td>8</td>
<td>50</td>
<td>3371 ± 52</td>
<td>80.1 ± 1.9</td>
<td>20.0 ± 0.8</td>
<td>40m; 60m</td>
<td>21.0 ± 0.3</td>
<td>2.4 ± 0.1</td>
<td>70.1 ± 1.1</td>
</tr>
<tr>
<td>Session Mean</td>
<td>3090 ± 89</td>
<td>76.2 ± 1.1</td>
<td>20.1 ± 0.2</td>
<td>35m; 50m</td>
<td>19.8 ± 0.4</td>
<td>2.0 ± 0.1</td>
<td>72.1 ± 0.5</td>
<td></td>
</tr>
</tbody>
</table>
5.5.2: Anthropometry and DXA

There were no significant changes within or between conditions for measurements of body mass, BMI and WHR (p>0.05; Table 5.1). TB-FM showed a significant relative (%) and absolute (kg) decrease within both SSG (-2.6 ±0.9%; P<0.001) and CYC (-2.9 ±1.1%; p<0.001), compared to a significant increase in the CON (3.0 ±1.2%; p=0.012) condition. TB-FFM (kg) showed a significant increase with SSG (2.0 ±0.7%; p=0.017), compared to CON (0.9 ±1.8%; p=0.195), whilst CYC showed no change (1.3 ±0.7%; p=0.10) compared to SSG (p=0.855) and CON (p=0.062) conditions.

5.5.3: Fasting blood chemistry

Fasting blood chemistry for inflammatory markers is provided in Table 5.3. CRP showed a significant decrease in SSG (-15.0 ±4.1%; p=0.008) and CYC (-14.0 ±5.4%; p=0.02) without change in CON (1.4 ±6.4%; p=0.94). IL-6 showed a decrease following SSG training (-30.6 ±6.1%; p=0.002), which was a significantly greater change than CYC (-10.5 ±5.1%; p=0.06) and CON (16.5 ±10.9%; p=0.27), respectively. Leptin decreased significantly with SSG training (-20.6 ±7.0%; P=0.01), without change in the CYC (3.8 ±8.9%; p=0.90) or CON (10.3 ±8.6%; p=0.46) conditions. No significant changes were evident within or between conditions in TNFα, IL-1β, IL-10, IL-1ra and adiponectin (p>0.05).
Table 5.3

Blood chemistry of inflammatory cytokines, CRP, leptin and adiponectin pre and post 8 weeks of small-sided games (n=10), cycle ergometry (n=11) and control (n=11). Data reported as mean ±SEM.

<table>
<thead>
<tr>
<th></th>
<th>Small-Sided Games</th>
<th>Cycle Ergometry</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre (mg L⁻¹)</td>
<td>Post (mg L⁻¹)</td>
<td>Pre (mg L⁻¹)</td>
</tr>
<tr>
<td>CRP</td>
<td>2.90 ± 0.19</td>
<td>2.45 ± 0.19</td>
<td>2.73 ± 0.30</td>
</tr>
<tr>
<td>IL-6 (pg mL⁻¹)</td>
<td>2.05 ± 0.25</td>
<td>1.35 ± 0.14</td>
<td>2.03 ± 0.23</td>
</tr>
<tr>
<td>TNF-α (pg mL⁻¹)</td>
<td>3.03 ± 0.46</td>
<td>2.63 ± 0.39</td>
<td>3.93 ± 0.64</td>
</tr>
<tr>
<td>IL-1β (pg mL⁻¹)</td>
<td>1.90 ± 0.24</td>
<td>1.61 ± 0.12</td>
<td>1.84 ± 0.13</td>
</tr>
<tr>
<td>IL-1ra (pg mL⁻¹)</td>
<td>281.4 ± 24.7</td>
<td>256.8 ± 23.9</td>
<td>274.0 ± 21.9</td>
</tr>
<tr>
<td>IL-10 (pg mL⁻¹)</td>
<td>0.44 ± 0.03</td>
<td>0.39 ± 0.02</td>
<td>0.55 ± 0.06</td>
</tr>
<tr>
<td>Leptin (ng mL⁻¹)</td>
<td>9146 ± 1217</td>
<td>6934 ± 736</td>
<td>11037 ± 1755</td>
</tr>
<tr>
<td>Adiponectin (ng mL⁻¹)</td>
<td>12749 ± 1561</td>
<td>11575 ± 1416</td>
<td>13876 ± 1173</td>
</tr>
</tbody>
</table>

CRP, C-reactive protein, IL, Interleukin, TNF, Tumor necrosis factor, ra, receptor agonist

* = Significant within group change (p<0.05); b = Significant change compared to control group (p<0.05)
5.5: Discussion

The inactive, middle-aged men recruited for the present study were classified as overweight, and had CRP and IL-6 concentrations which placed them at ‘high risk’ of developing metabolic and cardiovascular diseases (Alberti et al., 2006; Pradhan et al., 2001). This study demonstrates both SSG and CYC training in sedentary men are capable of inducing positive inflammatory and body composition adaptations, albeit to differing extents. Specifically, 8-weeks of SSG and CYC training increases sub-maximal power output and VO₂, decreases in TB-FM and systemic concentrations of CRP. Notably, additional benefits of SSG training include increases in TB-FFM and decreases in resting IL-6 and leptin concentrations.

The accumulation of body fat increases the chronic inflammatory state and may result in the development of metabolic abnormalities (Gleeson et al., 2011; Ouchi et al., 2011). In the present study both CYC and SSG training resulted in significant decreases in TB-FM in overweight middle-aged men. Previous observations report 12 weeks of SSG (soccer) training results in a significant decrement in TB-FM (3.0%) (Krustrup et al., 2010d). This response in SSG is comparable with cycle ergometry training of similar duration, which showed a 3.4% decrease in TB-FM (Donges et al., 2010). The present study showed comparative reductions in TB-FM (SSG at -2.6 ±0.9% and CYC at -2.9 ±1.1%), while SSG was the only training condition that increased TB-FFM (1.1 ±0.3 kg) compared to no change in CYC (0.7 ±0.4 kg) and CON (-0.8 ±0.6kg). These results are consistent with previous reports that show 12 weeks of SSG (soccer) training increases TB-FFM (1.7 ±0.4 kg) (Krustrup et al., 2009), while CYC training in sedentary individuals have shown minimal to no changes in TB-FFM (i.e. -0.6 kg and +0.7 kg) (Balducci et al., 2010; Donges et al., 2010; Samjoo, Safdar, Hamadeh, Raha, & Tarnopolsky, 2013). Eccentric loading is known to stimulate a higher rate of muscle protein synthesis and associated muscular hypertrophy,
especially compared to concentric contractions (Coffey & Hawley, 2007). Accordingly, it could be assumed the presence of eccentric contractions during running based demands of SSG, that are otherwise absent in CYC, could explain the increased TB-FFM in SSG.

Pro-inflammatory cytokines are secreted from adipose tissue, with an increase in TB-FM associated with an increase in pro-inflammatory cytokines i.e. CRP and IL-6 (Balducci et al., 2010; Fried et al., 1998; Petersen & Pedersen, 2005). Additionally, high rates of metabolic abnormalities are also associated with a low aerobic capacity and higher concentrations of CRP ($\geq 3.0 \text{ mg.L}^{-1}$) (Aronson et al., 2004a). Consequently, a sedentary lifestyle and excess accumulation of adipose tissue causes elevated levels of circulating inflammatory biomarkers (Fried et al., 1998; Petersen & Pedersen, 2005; You et al., 2013). Typically, chronic systemic concentrations of IL-6 stimulate an acute-phase response through the hepatic secretion of CRP (Gleeson et al., 2011). The chronic adaptations of CRP and IL-6 to continuous, aerobic exercise training (i.e. running, cycling) in obese participants remain equivocal (Kohut et al., 2006; Nicklas et al., 2004; Oberbach et al., 2006; You et al., 2013), while only one study has reported no change in CRP to SSG (soccer) training in a clinical cohort (although was inclusive of smokers, n=5; and those on medications n=2, statins; n=15, antihypertensive medications) (Andersen et al., 2010b). In the current study, CYC and SSG training reduced CRP by 14% and 15%, respectively, though SSG was the only condition to also reduce IL-6 concentration (30%). Accordingly, the present study supports previous findings which report a reduction in CRP concentration when fat mass is decreased (Aronson et al., 2004a; Donges et al., 2010). Moreover, high resting plasma concentrations of IL-6 are associated with lower muscle mass, hence, the increase in TB-FFM within the SSG condition could account for the decrease in IL-6 (Visser et al., 2002). Taken together, when compared to CYC and CON, SSG was the dominate training condition for reducing pro-inflammatory cytokines (IL-6 and
CRP) alongside increasing TB-FFM and thus representing a reduction in multiple risk-factors associated with the development of T2DM and CVD (Pradhan et al., 2001). The preservation of muscle mass through exercise training can up-regulate glucose regulatory mechanisms in skeletal muscle (i.e. GLUT4 translocation) which can further influence the pro- and anti-inflammatory state to ameliorate metabolic dysfunction (Gleeson et al., 2011; Petersen & Pedersen, 2005). As such, increases in muscle mass to SSG training may also have additional glucose regulatory effects at a systemic and skeletal muscle level; however, future research is required to test this hypothesis.

The first two cytokines produced in the pro-inflammatory cytokine cascade are TNF-α and IL-1β, and in addition to IL-6, stimulate the hepatic production of CRP (Petersen & Pedersen, 2005). Accordingly, chronic low-grade inflammation is typical of a two- to three-fold increase in systemic concentrations of TNF-α, IL-6, IL-1β, and/or CRP (Petersen & Pedersen, 2005). Despite a reduction in IL-6 and/or CRP in the present study, there were no changes in TNF-α or IL-1β. Previous research reporting the response of TNF-α and IL-1β with exercise training provide equivocal findings in either healthy or diabetic participants (Balducci et al., 2010; Kadoglou et al., 2007). The present results suggest that 8 weeks of exercise training resulting in reduced TB-FM were not adequate to mediate changes in TNF-α or IL-1β. Notably, the lack of change in IL-1β and TNF-α may be due to the resting concentration already being within the desirable ranges prior to training and thus limiting the potential for improvement. Recently, Fernández-Riejos et al. (Fernández-Riejos et al., 2010) demonstrated a dose-dependent leptin-induced stimulation of pro-inflammatory cytokines TNF-α and IL-6 by monocytes; although, many training studies have reported that leptin is only decreased in accordance with reduced fat-mass (Kraemer, Chu, & Castracane, 2002; Pasman, Westerterp-Plantenga, & Saris, 1998). In the present study, SSG was the only condition to reduce leptin
concentrations, even though both SSG and CYC reduced TB-FM. These results suggest that the change in leptin may have occurred independently to the relatively small changes in fat-mass and consequently, a myriad of other mechanisms such as increased muscle mass (Visser et al., 2002) glucose regulation (Silha et al., 2003), and/or changes in other pro-inflammatory cytokines (Fernández-Riejos et al., 2010) may be involved. Collectively, results of the current study report no change in TNF-α or IL-1β to exercise training, although, SSG was the dominant short-term training approach for reducing leptin when compared to cycle ergometry.

An imbalance of pro- and anti-inflammatory cytokines secreted from adipose tissue contributes to metabolic dysfunction (Arita et al., 1999; Ouchi et al., 2011). Adiponectin is another hormone secreted by adipocytes which stimulates an increase in the anti-inflammatory cytokines IL-10 and IL-1ra in monocytes and macrophages, while inhibiting systemic levels of IL-6 and TNF-α (Bouassida et al., 2010; Ouchi et al., 2011). The expression of adiponectin protects against metabolic and cardiovascular disorders and is decreased in plasma and adipose tissue in obese, compared to lean individuals (Carson et al., 2012; Ouchi et al., 2011). The current study showed no change in anti-inflammatory markers (adiponectin, IL-1ra and IL-10) in response to SSG or CYC training, despite the observed reductions in CRP and IL-6 noted earlier. Furthermore, previous reports suggest a strong positive correlation between the change in IL-1ra and change in muscle mass (Meier et al., 2002), although this was not evident in the current study, which showed no change in IL-1ra despite an increase in TB-FFM in the SSG condition. Moreover, previous exercise training studies report inconsistent results regarding adiponectin and IL-10 (Balducci et al., 2010; Bouassida et al., 2010; Kadoglou et al., 2007). These studies document an increase in IL-10 which was related to a reduction in fat-mass, and no change in adiponectin, or no change in
either variable in response to 6 and 12 months of aerobic exercise training (Balducci et al., 2010; Kadoglou et al., 2007), respectively. Although exercise alone has reported increases in adiponectin and IL-10, diet interventions in combination with exercise have been shown to increase these markers more robustly (Bouassida et al., 2010). As such, the current study shows 8-weeks of CYC or SSG does not stimulate an anti-inflammatory response and this may be due to the uncontrolled diet and calorie intake within the current study (Bouassida et al., 2010).

5.6.1: Conclusions

This study reports 8 weeks of SSG or CYC training reduced CRP concentration, and fat-mass. In addition, SSG training was the only condition to increase TB-FFM, and decrease pro-inflammatory markers, leptin and IL-6. These changes in body composition and pro-inflammatory markers were not associated with anti-inflammatory markers IL-10, IL-1ra or adiponectin, which showed no change in response to exercise training. These results demonstrate the importance of reducing fat-mass and increasing muscle mass for decreasing the concentration of pro-inflammatory markers. Collectively, while CYC training was effective at reducing several risk-factors, the differences between conditions show SSG to be a more effective training approach in reducing the inflammatory risk profile (CRP, IL-6 and leptin) and increasing muscle mass within sedentary, middle-aged men.
CHAPTER SIX

Rugby-specific small-sided games training is an effective alternative to continuous cycle ergometry for improving glucose regulation in middle-aged, inactive Caucasian men.

As accepted for publication

6.1: Abstract

**Aim:** The present study investigated whether SSG could be an effective alternative to CYC training at reducing clinical risk factors associated with the development of T2DM.

**Methods:** Thirty-three middle-aged (age 48.6 ±2.0 y), inactive men were randomised into a CYC (n=11), SSG (n=11), or CON (n=11) group. Participants in exercise groups trained 3d/wk for 8 weeks, while control participants maintained normal activity and dietary patterns. Groups were matched on exercise duration with CYC performed continuously and SSG split over four quarters interspersed with 2-min passive recovery periods. Both training programs were designed to elicit similar heart rates (~80-85% HR$_\text{max}$) and Rating of Perceived Exertion (RPE). Pre- and post-intervention testing included a dual-energy x-ray absorptiometry scan, a graded exercise test, a fasting 2 h oral glucose tolerance test (OGTT) and a resting muscle biopsy. Western blotting was used to assess the content of skeletal muscle proteins associated with mitochondrial biogenesis and glucose regulation.

**Results:** Both CYC and SSG increased VO$_2$ at 80% HR$_\text{max}$, and reduced glycated haemoglobin, glucose area under the curve (AUC; SSG, -2.3 ±0.8; CYC -2.2 ±0.5 mmolL$^{-1}$ (120 min)$^{-1}$; p<0.05), and total body fat-mass (SSG -2.6 ±0.9%; CYC -2.9 ± 1.1%) compared to no change in CON (p<0.05). Only SSG reduced insulin AUC (-30.4 ±12.9 µIU·mL$^{-1}$ (120 min)$^{-1}$; p<0.05) and increased total body fat-free mass (1.1 ±0.4 kg; p<0.05), with no change in CYC or CON (P>0.05). No significant differences were found within or between conditions for total protein content of PGC-1α, SIRT1, p53, GLUT4, Akt, MEF2A, Tfam, NRF1, NRF2 or mitochondrial complexes I-V (p>0.05).

**Conclusion:** Intermittent exercise training via SSG is an effective alternative to continuous cycling for improving metabolic risk-factors associated with the prevention of T2DM. Despite such positive adaptations in clinical risk factors, there were no changes in the content of skeletal muscle proteins associated with glucose regulation and mitochondrial biogenesis.
6.2: Introduction

Physical inactivity is strongly associated with the development of chronic diseases such as T2DM (Durstine et al., 2012). Therefore, regular exercise can be a potent primary prevention strategy against chronic disease development that can assist to counter the economic and social repercussions of these diseases (Durstine et al., 2012). The benefits of exercise for chronic disease prevention have traditionally been associated with systemic adaptations and improvements in clinical risk factors (i.e. fat-mass, muscle mass, aerobic capacity, lipid profile) associated with glucose regulation and/or insulin sensitivity (Alberti et al., 2006; Durstine et al., 2012; Laaksonen et al., 2002). In part, these clinical benefits have also been attributed to metabolic and molecular remodelling within skeletal muscle (Egan & Zierath, 2013).

To date, the majority of research has focused on the health benefits of continuous, aerobic-based exercise training, such as walking, running and cycling (Egan & Zierath, 2013). More recently, prolonged intermittent exercise, such as football-specific SSG training, has the potential to improve motivation and compliance compared with continuous aerobic exercise (Krustrup et al., 2010a). Football-specific (futsal) SSG training has also been associated with positive adaptations on measures shown to influence glucose regulation and insulin sensitivity (i.e. body composition, aerobic capacity, blood pressure, capillary and density fibre type), which are either comparable to, or better than, continuous, aerobic exercise training (Andersen et al., 2014; de Sousa et al., 2014; Knoepfli-Lenzin et al., 2010; Krustrup et al., 2010a; Krustrup et al., 2010d; Serpiello et al., 2014). While this suggests that SSG training should be effective for improving glucose regulation and insulin sensitivity, there are no published data directly examining this hypothesis.
Insulin resistance has also been linked with mitochondrial dysfunction and the reduced content of key regulatory proteins, which have been shown to increase with aerobic or high-intensity, low-volume, sprint interval training (SIT) (Baar et al., 2002). Recently, SSG training has been reported to increase several factors known to be associated with increases in the content of skeletal muscle proteins important for mitochondrial biogenesis (Egan & Zierath, 2013). These factors include increases in VO$_2$max, citrate synthase activity, number of capillaries per muscle fibre, and muscle fibre area (Krstrup et al., 2010b). However, to date, there has been no published research directly investigating the effects of SSG training on the content of skeletal muscle proteins associated with mitochondrial biogenesis and improved glucose metabolism.

It has been proposed that exercise training improves insulin sensitivity and glucose uptake, which can be partly explained by increased content of GLUT4 and Akt (Gonzalez & Mcgraw, 2006; Goodyear & Kahn, 1998). Exercise training has also been reported to increase the content of PGC-1α, which in turn regulates mitochondrial transcription factors, function and overall protein content, with over expression associated with improved enzyme activity, insulin sensitivity and aerobic capacity (Bartlett et al., 2012). Additional proteins associated with PGC-1α and mitochondrial functioning are SIRT1 and p53 (Bartlett et al., 2012). Increased SIRT1 activity has been implicated in the induction of mitochondrial biogenesis following exercise training (Gurd et al., 2010). While the acute exercise response of p53 has been reported (Bartlett et al., 2012), the response to exercise training in humans is unknown. Since SSG training has been reported to improve aerobic capacity, body composition and skeletal muscle structure, it may also improve the content of proteins associated with mitochondrial biogenesis and cellular glucose uptake, so as to improve glucose tolerance.
Understanding the effects of different types of exercise on skeletal muscle regulatory proteins implicated in glucose control and mitochondrial biogenesis, and how they interact with systemic changes in glucose metabolism, may assist to improve preventative and therapeutic strategies for inactive populations. Therefore, the first aim of this study was to compare the effects of continuous cycling and SSG training on risk factors (body composition, aerobic capacity and strength) and indicators (blood glucose metabolism and regulation) associated with the development of T2DM. The second aim was to investigate changes within and between the respective conditions for the content of skeletal muscle proteins that have been associated with glucose control and mitochondrial biogenesis. It was hypothesised that SSG would be an effective alternative to continuous (i.e. cycle ergometry) training for inactive, middle-age men, capable of eliciting positive changes in clinical risk-factors and skeletal muscle proteins associated with the prevention of T2DM.

6.3: Methods

6.3.1: Participant recruitment

Thirty-three men (Table 6.1; age 48.6 ±2.1 y; stature 176.7 ±1.8 cm; mass 89.8 ±3.7 kg) were recruited and randomly assigned to a stationary cycle ergometry (CYC, n=11), a SSG (n=11), or control (CON, n=11) group (Figure 6.1). Participant allocation to conditions was conducted through block randomization (Microsoft Office, excel 2010) in groups of 3 by a research assistant. Participants were recruited through verbal communication and newspaper advertisements within a regional Australian community. Based on verbal communication and the self-reporting of activity patterns, participant recruitment ensured the inclusion of participants who were non-smokers, inactive for a minimum of 12 months (i.e. no regular pattern of planned strenuous activity or exercise of >60 min per week), with no clinically
diagnosed cardiovascular or metabolic disorders and taking no prescribed medications. Participants with orthopedic limitations were excluded from the study. Neither the researchers nor participants were blinded to group allocation during pre and post-intervention.

Prior to participant recruitment and testing procedures, Human Ethics clearance was obtained from Charles Sturt University Human Research Ethics Committee (Protocol number: 2011/113; approved September 2011) and participants attended an information and familiarization session where verbal and written informed consent for all testing and training procedures was obtained. This study was registered for inclusion in the Australian New Zealand Clinical Trial Registry and was allocated the clinical trial registration number: ACTRN12613000874718. Clinical trial registration is not an ethical requirement of the University, thus, registration occurred after participant enrollment due to delayed awareness of required clinical registration. The authors confirm that all ongoing and related trials for this intervention are registered. Complete data sets (raw data obtained from each individual participant) from this study will be provided upon requests made to the corresponding author.
Table 6.1

Mean ± SEM participant characteristics, anthropometry, body composition, aerobic capacity and strength pre and post 8 weeks of control (n=11), continuous cycle ergometry (n=11) or small-sided games (n=10).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Cycle Ergometry</th>
<th>Small-Sided Games</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>Age (y)</td>
<td>49.2 ± 2.12</td>
<td>-</td>
<td>49.5 ± 2.0</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.78 ± 0.02</td>
<td>-</td>
<td>1.76 ± 0.01</td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td>92.6 ± 3.4</td>
<td>92.8 ± 3.3</td>
<td>90.3 ± 3.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.5 ± 1.0</td>
<td>29.4 ± 0.9</td>
<td>29.1 ± 1.1</td>
</tr>
<tr>
<td>Waist girth (cm)</td>
<td>99.1 ± 2.6</td>
<td>98.5 ± 2.6</td>
<td>96.9 ± 2.6</td>
</tr>
<tr>
<td>WHR</td>
<td>0.96 ± 0.01</td>
<td>0.95 ± 0.01</td>
<td>0.95 ± 0.02</td>
</tr>
<tr>
<td>DXA analysis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TB-FM (kg)</td>
<td>27.1 ± 2.8</td>
<td>27.9 ± 2.6^a</td>
<td>26.7 ± 2.5</td>
</tr>
<tr>
<td>TB-FM (%)</td>
<td>28.5 ± 2.1</td>
<td>29.5 ± 2.1^a</td>
<td>28.9 ± 1.9</td>
</tr>
<tr>
<td>TB-FFM (kg)</td>
<td>62.6 ± 1.9</td>
<td>61.8 ± 2.1</td>
<td>60.3 ± 1.9</td>
</tr>
<tr>
<td>IA-FM (kg)</td>
<td>2.6 ± 3.0</td>
<td>2.7 ± 0.3^a</td>
<td>2.7 ± 0.3</td>
</tr>
<tr>
<td>GXT to 80% HRmax</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VO₂ (mL kg⁻¹ min⁻¹)</td>
<td>26.5 ± 1.6</td>
<td>25.7 ± 1.4</td>
<td>24.2 ± 1.2</td>
</tr>
<tr>
<td>VO₂ (L min⁻¹)</td>
<td>2.4 ± 0.1</td>
<td>2.3 ± 0.1</td>
<td>2.2 ± 0.2</td>
</tr>
<tr>
<td>Workload (Watts)</td>
<td>227 ± 10</td>
<td>230 ± 11</td>
<td>207 ± 10</td>
</tr>
<tr>
<td>Duration (min)</td>
<td>7.5 ± 0.4</td>
<td>7.6 ± 0.4</td>
<td>7.0 ± 0.4</td>
</tr>
<tr>
<td>Strength (3RM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leg Press (kg)</td>
<td>164 ± 16</td>
<td>164 ± 15</td>
<td>182 ± 12</td>
</tr>
<tr>
<td>Chest Press (kg)</td>
<td>72 ± 6</td>
<td>71 ± 7</td>
<td>62 ± 3</td>
</tr>
</tbody>
</table>

BMI, Body mass index; WHR, Waist to hip ratio; DXA, Dual-energy x-ray absorptiometry; TB-FM, Total body-fat mass; TB-FFM, Total body-fat free mass; IA-FM, Intra-abdominal fat mass; GXT, Graded exercise test; HRmax, Maximal heart rate; VO₂, Oxygen consumption; 3RM, 3 repetitions maximal.

^a = Significant within group change (p<0.05); ^b = Significant change compared to control group (p<0.05); ^c = Significant change compared to cycle ergometry group (p<0.05).
6.3.2: Overview

Participants attended two pre- and two post-intervention testing sessions. The first session comprised of resting blood pressure, a sub-maximal GXT and a 3-repetition maximum (3RM) chest press and leg press. After a minimum of 72 h recovery from the previous testing session, participants returned for a second session that included anthropometric measurements, a DXA scan, a resting muscle biopsy and a 2-h OGTT. The exercise interventions consisted of 8 weeks of SSG (modified rugby) or CYC training. Participants were required to attend at least 90% of all training sessions. Given the different exercise modes, the challenge of matching external training load or metabolic cost is acknowledged. However, in an attempt to match training load between conditions, the respective training programs were designed to elicit similar internal training loads as determined from ~80-85% age-predicted (220-age) HRmax and RPE (Hill-Haas et al., 2011).

6.3.3: Nutritional and physical activity standardisation

Prior to the start of all testing sessions participants refrained from any physical activity for 72 h, and the consumption of alcohol and caffeine for 24 h. Participants documented diet and physical activity patterns 24 h prior to pre-intervention testing. This document was photocopied and issued to the participants to ensure diet was replicated for the 24-h period prior to post-intervention testing. Prior to participation in the study, all participants were informed (verbal and written) of the importance of maintaining their normal dietary and physical activity patterns throughout the 8-week training period with lack of compliance resulting in exclusion from the program. Participants were required to maintain food and beverage type and timing of consumption, including cooking preparation and portion size. Physical activity was standardised to ensure all participants in all conditions did not change planned or incidental physical activity during the 8-week intervention.
6.3.4: Cycle ergometry training

Participants performed CYC training (Monark 828E, Monark Exercise AB, Varburg, Sweden) for three supervised sessions per week (details of training load progression are presented in Table 6.2). To quantify external training load, kp, rpm and total distance (km) were recorded at 5-min intervals during each session, with training load and progression manipulated through alternate increases in session duration and resistance (kp). Internal training load was quantified via HR (Vantage NV, Polar, Kempele, Finland) and recorded at 5-min intervals, while a session-RPE (Borg’s 6-20 scale) was obtained at the conclusion of each training session (Herman et al., 2006).
Table 6.2
Mean ± SEM of session training load over 8-weeks within the continuous cycle ergometry and small-sided games conditions.

<table>
<thead>
<tr>
<th>Week</th>
<th>Exercise Duration (min)</th>
<th>Power output (W)</th>
<th>Resistance (kp)</th>
<th>Cadence (RPM)</th>
<th>Mean Heart rate (%max)</th>
<th>RPE (AU)</th>
<th>Total Distance (m)</th>
<th>Mean speed (m/min)</th>
<th>Peak Speed (km/h)</th>
<th>Field Size</th>
<th>Mena Heart rate (%max)</th>
<th>RPE (AU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40</td>
<td>100 ± 6</td>
<td>1.5 ± 0.1</td>
<td>72.1 ± 1.3</td>
<td>80.5 ± 0.5</td>
<td>11.9 ± 0.2</td>
<td>2563 ± 53</td>
<td>71.5 ± 1.6</td>
<td>20.0 ± 0.6</td>
<td>25m; 40m</td>
<td>86.0 ± 1.0</td>
<td>12.9 ± 0.2</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>117 ± 6</td>
<td>1.7 ± 0.1</td>
<td>73.7 ± 1.5</td>
<td>83.9 ± 0.8</td>
<td>12.1 ± 0.2</td>
<td>2642 ± 74</td>
<td>73.4 ± 2.1</td>
<td>18.6 ± 0.5</td>
<td>25m; 40m</td>
<td>85.8 ± 0.9</td>
<td>12.2 ± 0.3</td>
</tr>
<tr>
<td>3</td>
<td>45</td>
<td>121 ± 8</td>
<td>1.9 ± 0.1</td>
<td>76.0 ± 1.2</td>
<td>85.4 ± 0.9</td>
<td>12.3 ± 0.2</td>
<td>2856 ± 59</td>
<td>71.3 ± 1.5</td>
<td>20.0 ± 0.6</td>
<td>25m; 40m</td>
<td>86.5 ± 1.0</td>
<td>12.0 ± 0.2</td>
</tr>
<tr>
<td>4</td>
<td>45</td>
<td>132 ± 7</td>
<td>2.2 ± 0.1</td>
<td>73.7 ± 1.0</td>
<td>84.9 ± 0.9</td>
<td>12.6 ± 0.1</td>
<td>3014 ± 63</td>
<td>75.1 ± 1.6</td>
<td>21.2 ± 0.6</td>
<td>35m; 50m</td>
<td>85.3 ± 0.8</td>
<td>12.1 ± 0.2</td>
</tr>
<tr>
<td>5</td>
<td>45</td>
<td>148 ± 6</td>
<td>2.2 ± 0.1</td>
<td>72.3 ± 1.2</td>
<td>85.6 ± 0.9</td>
<td>12.8 ± 0.1</td>
<td>3117 ± 62</td>
<td>77.8 ± 1.5</td>
<td>20.2 ± 0.7</td>
<td>35m; 50m</td>
<td>83.3 ± 1.0</td>
<td>12.3 ± 0.2</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>146 ± 6</td>
<td>2.2 ± 0.1</td>
<td>70.1 ± 1.0</td>
<td>86.0 ± 0.8</td>
<td>12.8 ± 0.2</td>
<td>3541 ± 70</td>
<td>78.8 ± 1.6</td>
<td>20.9 ± 0.6</td>
<td>35m; 50m</td>
<td>85.8 ± 1.1</td>
<td>12.7 ± 0.2</td>
</tr>
<tr>
<td>7</td>
<td>50</td>
<td>161 ± 3</td>
<td>2.4 ± 0.1</td>
<td>69.3 ± 0.9</td>
<td>86.4 ± 1.0</td>
<td>12.8 ± 01</td>
<td>3673 ± 59</td>
<td>82.0 ± 1.2</td>
<td>20.3 ± 0.7</td>
<td>40m; 60m</td>
<td>84.2 ± 0.9</td>
<td>12.2 ± 0.2</td>
</tr>
<tr>
<td>8</td>
<td>50</td>
<td>165 ± 3</td>
<td>2.4 ± 0.1</td>
<td>70.1 ± 1.1</td>
<td>84.2 ± 1.1</td>
<td>12.4 ± 0.1</td>
<td>3371 ± 52</td>
<td>80.1 ± 1.9</td>
<td>20.0 ± 0.8</td>
<td>40m; 60m</td>
<td>84.7 ± 1.0</td>
<td>12.5 ± 0.2</td>
</tr>
<tr>
<td>Session Mean</td>
<td>136 ± 4</td>
<td>2.0 ± 0.1</td>
<td>72.1 ± 0.5</td>
<td>84.6 ± 0.4</td>
<td>12.5 ± 0.1</td>
<td></td>
<td>3090 ± 89</td>
<td>76.2 ± 1.1</td>
<td>20.1 ± 0.2</td>
<td>35m; 50m</td>
<td>85.2 ± 0.3</td>
<td>12.4 ± 0.1</td>
</tr>
</tbody>
</table>

No significant differences were evident between conditions for heart rate or RPE (p>0.05).
6.3.5: Small-sided games training

The SSG group performed three supervised sessions per week of modified rugby league (most popular football code in this geographical region). The modified rugby session was played under touch football rules. The rules allowed each team six ‘plays’ while in possession of the ball; each play required players to pass the ball backwards to an ‘on side’ team member with the aim to score at opposing ends of the field. Defending players were required to touch their opponent with one hand. Following a successful touch, game play would restart with a ‘play the ball’, at this time requiring the line of defending players to be 5 m from the position of each ‘play the ball’ (Kennett et al., 2012). Each training session was comprised of four quarters, interspersed by 2-min passive recovery periods. See Table 6.2 for details of progression and training load. To quantify external training load, a GPS device (SPI-Pro, 1 Hz, GPSports, Canberra, ACT, Australia) was worn in a customised harness between the scapulae to quantify total distance (m), mean speed (m min\(^{-1}\)) and peak speed (km h\(^{-1}\)) of each training session. During the 8 weeks, training load progressively increased via manipulation of session duration and field size, including consistent game rules, verbal feedback and player numbers at 5 v 5 or 6 v 6 (depending on participant availability). Heart rate was recorded during, and session-RPE was obtained at the conclusion of all training sessions, to determine internal training load (Herman et al., 2006).

6.3.6: Control condition

The CON group completed all pre- and post-intervention testing sessions and was required to continue their normal diet and physical activity patterns over the 8-week intervention period. Participants were provided with a diet and physical activity journal to document any changes. Prior to post-intervention testing each journal was reviewed by the chief investigator to ensure individual conformity to the control condition.
6.3.7: Anthropometry and DXA

All testing procedures were conducted at a standardised time between 0600 and 0900 h. Anthropometric measures included stature (Stadiometer, CSU, Bathurst, Australia), body mass on calibrated scales (HW 150 K; A&D, Bradford, MA, USA), waist and hip girths (EC P3 steel tape Sydney, Australia) using standard techniques (Marfell-Jones et al., 2012). These measures were used to calculate BMI and the WHR (Marfell-Jones et al., 2012). Manual blood pressure was obtained with an aneroid sphygmomanometer and cuff (Welch-Allyn, Arden, North Carolina, USA) and expressed as the mean of three measurements after the participant had been seated for five minutes. A supine, whole-body DXA scan (XR800, Norland, Cooper Surgical Company, USA) was conducted with the scanning resolution set at 6.5 x 13.0 mm, and the scanning speed set at 130 mm·s⁻¹. Scans were analysed (Illuminatus DXA, ver. 4.2.0, USA) for TB-FM, TB-FFM and IA-FM. Analysis of IA-FM was performed with the creation of a 10 cm region of interest standardised across all participants according to previously outlined procedures, with a reported coefficient of variation for fat-mass of 2.6% (Hill et al., 2007).

6.3.8: Graded exercise test

Aerobic capacity measures were obtained via a GXT to determine sub-maximal VO₂. A sub-maximal GXT was used in preference to maximal testing to minimise associated risks in middle-aged, inactive men (Wallman & Campbell, 2007). The GXT was performed on an electronically-braked cycle ergometer (Excalibur Sport, LODE BV, Groningen, The Netherlands), commencing at 25 W and increasing by 25 W every min. Pulmonary gas exchange was measured by determining O₂ and CO₂ concentrations and ventilation to calculate VO₂ using a metabolic gas analysis system (Parvo Medics, True2400, East Sandy, UT, USA). The flow meter was calibrated using a three-litre syringe, while gas analysers
were calibrated for fractional gas concentration with a gravimetric gas mixture of known concentrations (CO₂, 4.1 (0.1)%; O₂, 15.7 (0.2)%), in accordance with the manufacturer’s instructions. Heart rate was recorded each minute throughout the protocol, and participants exercised until attainment of 80% HRₘₐₓ.

6.3.9: Maximal strength testing

Following a 30-min recovery period from the GXT, participants completed a 3RM test. Testing procedures determined upper- and lower-body strength via seated chest press and leg press (Panatta Sport, Apiro, Italy), respectively. Participants attempted ascending resistances, separated by a 3-min recovery period, until the determination of upper- and lower-body 3RM. Individual seating positions of head rest, back rest and seat height were recorded and replicated during post-intervention testing.

6.3.10: Oral glucose tolerance test

Following overnight fast (10-12 h) participants presented to the laboratory for a 2-h OGTT. Participants were cannulated in the medial cubital vein for venous blood sampling during the OGTT. Following the collection of a fasting venous blood sample each participant ingested a standardised 75 g glucose solution within a 5-min period and remained rested for the remaining blood collections at 30, 60, 90 and 120 min post glucose ingestion.

6.3.11: Venous blood collection and analysis

For all time-points venous blood samples were collected in SST (5 mL; cholesterol, triglycerides and insulin) and fluoride oxalate (4 mL; glucose) tubes. All samples were centrifuged at 3500 rpm for 15 min at 4°C. Aliquots were frozen immediately at -20°C until
analysis. At rest whole blood was refrigerated (4°C) in an EDTA tube (4 mL) for a maximum of 6 h until analysis of HbA1c.

Fasting venous blood samples were analysed for total cholesterol (Enzymatic method and polychromatic endpoint technique), HDL (Accelerator selective detergent methodology), LDL (Friedwald Equation), triglycerides (Enzymatic method and biochromatic endpoint technique; Dimension Xpand Plus, Siemens Healthcare Diagnostics, Sydney, Australia) and HbA1c (%A1c) (High-performance liquid chromatography: Bio-Rad Variant, Bio-Rad Laboratories, Sydney, Australia). Samples collected from the OGTT were also analysed for glucose (ABL825 Flex Analyser, Radiometer Medical ApS, Bronshoj, Denmark) and insulin (Solid-phase chemiluminescent enzyme immunometric assay: Immulite 2000, Siemens Healthcare Diagnostics, Los Angeles, CA, USA) with an intra and inter-assay coefficients of variation between 4.0-7.4%. As an indication of HOMA-IR was calculated based on (fasting insulin x fasting glucose)/22.5 (Matthews et al., 1985). For the analysis of blood glucose metabolism, glucose and insulin AUC over the 2 h OGTT was calculated using previously validated methods (Allison, Paultre, Maggio, Mezzitis, & Pi-Sunyer, 1995) and estimated insulin sensitivity was calculated through the previously validated Matsuda-ISI model (Matsuda & Defronzo, 1999).

6.3.12: Muscle biopsy collection

For both pre and post-intervention muscle biopsies, it was ensured that participants were in a fasted state (10-12 h) and refrained from physical activity for 72 h prior to collection. Final training sessions were staggered across all groups to ensure accurate timing of the 72 h post-training muscle samples. After administration of a local anaesthetic (2% plain Lignocaine) a muscle biopsy was obtained from the lateral portion of the m. vastus lateralis of each
participant. Using a 5-mm Bergstrom needle biopsy an ~100 mg sample was obtained, blotted on filter paper, removed of fat and connective tissue, frozen in liquid nitrogen and stored at -80°C until further analysis. Samples were analysed for total protein content of PGC-1α (Calbiochem ST1202), NRF1 (Abcam ab34682), NRF2 (Santa Cruz sc-22810), Tfam (Abcam ab47517), mitochondrial complex I-V (Mitoprofile Human Total OXPHOS antibody cocktail, Mitosciences ab110411), MEF2A (Abcam ab87975), SIRT 1 (Cell Signaling 8469), GLUT4 (Millipore CBL242), p53 (Cell Signaling 2527), AKT (Cell Signaling 9272) and α-tubulin (Cell Signaling 2125), which was used as a loading control.

6.3.13: Western blotting analyses

Approximately 20 mg of frozen muscle was homogenised in 400 µL of ice-cold lysis buffer (50 mM Tris-HCl (pH 7.4), 1% Triton X-100, 0.1% SDS, 1µg.mL⁻¹ Aprotinin and Leupeptin, 1 mM Benzamidine, 1 mM NaF, 150 mM NaCl, 1mM EDTA, 5 mM Na-pyrophosphate, 1 mM DTT, 1 mM PMSF and 1 mM Na₃ VO₄). Samples were homogenised followed by end-over-end rotation for 60 min at 4°C. Homogenates were centrifuged at 15,000 g for 10 min at 4°C and supernatant was collected. The protein content of the supernatant was determined with a Bradford assay using a protein assay dye reagent and bovine serum albumin (BSA) as the standard. Each sample was diluted with equal volume 2X Laemmli buffer (125 mM Tris-HCl (pH 6.8; 4% SDS, 20% glycerol, 0.015% Bromophenol Blue) and β-mercaptoethanol (10%). For each blot an internal standard was loaded along with 10-25 µg of protein for each sample and separated in Tris-glycine running buffer using self-cast stacking 4% and 8-12% resolving gels. In transfer buffer, gels were transferred wet onto PVDF membranes for 90 min at 100 V. Membranes were blocked at room temperature (RT) by incubating for 1 h in 5% fat-free milk and Tris buffered saline 0.1% Tween-20 (TBST). Membranes were washed 3 x 5 min in TBST and then incubated with primary antibodies (dilutions based on the
manufacturer’s instructions) in 3% fat-free milk or fatty acid-free BSA overnight (16 h) at 4°C.

The following morning, membranes were washed for a further 3 x 5 min in TBST and incubated with anti-species horseradish peroxidise-conjugated secondary antibody (1:10000 dilutions) in 1% fat-free milk for 90 min at room temperature. After a further 3 x 5-min washes in TBST, membranes were exposed to a chemiluminescence liquid (2.5 mM Luminol, 400 µM p-coumaric acid, 100 µM Tris (pH 8.5), 5.4 mM H2O2) for 5 min. Membranes were visualised using a Versa Doc 4000 MP imaging system and band densities were determined using Quality One image-analysis software, Bio-Rad. Pre and post samples for each participant and a participant representative from each training group were run in the same gel. Raw densitometry data were used for statistical analysis, and for graphical purposes fold change relative to pre-training values are displayed in Figures 6.1 and 6.2.

6.3.14: Statistical analyses
All data are reported as mean ± SEM, and all statistical analyses were conducted on raw data points and the change in raw data values. Non-normally distributed variables (PGC-1α, SIRT1, p53, GLUT4, AKT, MEF2A, Ttam, NRF1, NRF2 and mitochondrial complexes I-V) were log transformed prior to all analyses. A one-way repeated measure ANOVA (pre to post intervention) was used to compare baseline variables between conditions and the effects of each intervention for all measured variables with Tukey’s HSD post-hoc test. Two-way repeated measures ANOVA (pre to post intervention x 5 time points of glucose load) was used to assess the effect of each intervention on glucose and insulin. When a significant condition x time interaction occurred a post-hoc paired sample t-tests were used to determine where any difference lay pre- to post-intervention within each group. Significance was
accepted at P≤0.05. All statistical analyses were performed using PASW™ for MS-Windows version 20.0 (Statistical Package for the Social Sciences, Chicago, IL, USA).

6.4: Results

6.4.1: Training load and compliance

Participant numbers for completion of the study were CON (n=9), CYC (n=11) and SSG (n=10; Figure 6.1). Two participants dropped out of the control group (no reason provided) and one participant could not complete the SSG training due to a knee injury sustained during training (week 3). There were no significant differences in attendance to training sessions between the groups with a mean of 91 ±2% for SSG and 95 ±2% for CYC (p=0.127). Training intensity was comparable between conditions as represented by mean % HR_max (SSG, 85.3 ±1.1%; CYC, 84.5 ±1.3%; p=0.641) and session-RPE (SSG, 11.3 ±0.4 AU; CYC 12.0 ±0.2 AU; p=0.112; Table 2).

6.4.2: Body composition

Results showed no significant changes within or between conditions for measurements of body mass, BMI, waist girth and WHR (p>0.05; Table 6.1). Changes within and between conditions for TB-FM, TB-FFM, and IA-FM are provided in Table 1. Relative and absolute TB-FM decreased significantly within SSG (-2.6 ±0.9% and -0.8 ±0.9 kg; p=0.001) and CYC (-2.9 ± 1.1% and -0.7 ±0.8 kg; p=0.001), compared to a significant increase in the CON (3.0 ±1.2% and 0.8 ±1.3 kg; p=0.012). Total body fat-free mass increased significantly within SSG (1.1 ±1.2 kg; p=0.017), compared to CON (-0.8 ±1.9 kg; p=0.195), while CYC showed no change (0.8 ±1.4 kg; p=0.10) relative to SSG (p=0.855) or CON (p=0.062). Comparisons
between conditions show IA-FM in SSG (-51 ±106 g; p=0.034) and CYC (-132 ±128 g; p=0.001) significantly decreased, compared to an increase in CON (95 ±140 g; p=0.048).

6.4.3: Oral glucose tolerance test and fasting blood chemistry

Results from the OGTT, estimated insulin sensitivity, and fasting blood glucose, insulin and cholesterol are provided in Table 6.3. There were no significant changes within or between conditions for fasting blood glucose, insulin and cholesterol values (p>0.05). A significant decrease in HbA1c (%A1c) was evident in SSG (p=0.001) and CYC (p=0.02) compared to CON (p=0.80). A significant increase in estimated insulin sensitivity (Matsuda ISI) was evident in both SSG (3.1 ±0.8 µlU⋅mL⁻¹, mg⋅mL⁻¹; p=0.004) and CYC (2.7 ±1.3 µlU⋅mL⁻¹, mg⋅mL⁻¹; p=0.05), with no significant change in CON (-2.7 ±1.4 µlU⋅mL⁻¹, mg⋅mL⁻¹; p=0.084).

Glucose AUC decreased by -2.3 ±0.8 mmol⋅L⁻¹ (120 min)¹ in SSG (p=0.016), -2.2 ±0.5 mmol⋅L⁻¹ (120 min)¹ in CYC (p=0.001) and increased significantly in the CON group (0.8 ±0.9 mmol⋅L⁻¹ (120 min)¹; p=0.016) with a significant between-group interaction (p=0.008).

Insulin AUC was significantly decreased in SSG (-30.4 ±12.9 µlU⋅mL⁻¹ (120 min)¹; p=0.003) relative to CON (34.3 ±14.39 µlU⋅mL⁻¹ (120 min)¹; p=0.003), whilst CYC showed no significant change (-4.0 ±9.5 µlU⋅mL⁻¹ (120 min)¹; p=0.69) relative to SSG (p=0.291) or CON (p=0.085) conditions.

6.4.4: Oxygen consumption and strength

Changes to upper- and lower-body strength, and aerobic capacity variables (VO₂, test duration and power output at 80% HRₘₐₓ), are provided in Table 6.1. There was a significant increase in VO₂ at 80% HRₘₐₓ in both CYC (19.1 ±7.1%; p=0.002) and SSG (18.9 ±6.0%; p=0.001), but not CON (-3.5 ±1.7%; p=0.09). This corresponded to a significant increase in test duration of 30.2 ±8.5% (P=0.001) in CYC and 27.8 ±8.5% (p=0.004) in SSG, but not CON (1.1 ±2.1%; p=0.56). Power output (Watts) increased significantly in the CYC (21.2
±7.2%; p=0.009) and SSG (25.8 ±5.1%; p=0.001) groups, with no change in CON (0.9 ±1.8%; p=0.588). Chest press showed no significant changes within or between all conditions (p=0.388). Leg press increased significantly in SSG (16 ±5%; p=0.061), which was significantly different to the CYC (5 ±2%; p=0.040) and CON (1 ±2%; p=0.003).
**Table 6.3**

Mean ± SEM fasting blood chemistry and glucose tolerance test pre and post 8 weeks of control (n=11), continuous cycle ergometry (n=11) or small-sided games (n=10).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Cycling Ergometry</th>
<th>Small-Sided Games</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>Glucose (mmol·L⁻¹)</td>
<td>4.8±0.3</td>
<td>4.8±0.3</td>
<td>4.8±0.2</td>
</tr>
<tr>
<td>Insulin (µU·mL⁻¹)</td>
<td>8.8±2.0</td>
<td>10.1±2.5</td>
<td>8.5±2.4</td>
</tr>
<tr>
<td>Glucose AUC (mmol·L⁻¹(120 min)⁻¹)</td>
<td>12.3±0.5</td>
<td>13.1±0.9</td>
<td>14.1±1.1</td>
</tr>
<tr>
<td>Insulin AUC (µU·mL⁻¹(120 min)⁻¹)</td>
<td>111.6±22.3</td>
<td>140.7±25.7</td>
<td>108.5±23.6</td>
</tr>
<tr>
<td>Matsuda ISI (µU·mL⁻¹, mg·mL⁻¹)</td>
<td>9.4±2.3</td>
<td>6.7±1.3</td>
<td>8.5±2.1</td>
</tr>
<tr>
<td>HOMA-IR (µU·mL⁻¹, mmol·L⁻¹)</td>
<td>2.0±0.5</td>
<td>2.3±0.7</td>
<td>1.9±0.6</td>
</tr>
<tr>
<td>HbA1c (%A1c)</td>
<td>5.5±0.1</td>
<td>5.5±0.1</td>
<td>5.7±0.2</td>
</tr>
<tr>
<td>Total Cholesterol (mmol·L⁻¹)</td>
<td>5.1±0.3</td>
<td>5.1±0.2</td>
<td>5.3±0.2</td>
</tr>
<tr>
<td>HDL (mmol·L⁻¹)</td>
<td>1.3±0.1</td>
<td>1.2±0.1</td>
<td>1.3±0.1</td>
</tr>
<tr>
<td>Triglycerides (mmol·L⁻¹)</td>
<td>1.1±0.2</td>
<td>1.3±0.1</td>
<td>1.3±0.2</td>
</tr>
<tr>
<td>Hazard Ratio (Total : HDL)</td>
<td>3.9±0.4</td>
<td>4.2±0.3</td>
<td>4.3±0.3</td>
</tr>
</tbody>
</table>

AUC, Area under the curve; Matsuda ISI, Estimate of insulin sensitivity; HOMA-IR, Glucose homeostasis - insulin resistance; HDL, High density lipoproteins.

* a = Significant within group change (p<0.05); b = Significant change compared to control group (p<0.05).
6.4.5: Skeletal muscle protein content

Results for PGC-1α, SIRT-1, p53, GLUT4 and Akt are shown in Figure 6.1. Mitochondrial complexes I-V are reported in Figure 6.2. All data are reported as fold change relative to the pre-training values. There were no significant changes within or between conditions in any of the respective proteins as a result of the 8-week intervention (p>0.05). The transcription factors MEF2A (CON, 0.69 ±0.24; CYC, 0.90 ±0.22; SSG, 1.06 ±0.33 AU; p>0.05), Tfam (CON, 0.82 ±0.17; CYC, 0.81 ±0.24; SSG, 0.74 ±0.16 AU; p>0.05), NRF1 (CON, 0.98 ±0.23, CYC, 1.12 ±0.20; SSG, 0.69 ±0.12 AU; p>0.05) and NRF2 (CON, 0.84 ±0.35; CYC, 1.26 ±0.24; SSG, 0.78 ±0.22 AU; p>0.05) also showed no significant changes in response to the exercise training program (p>0.05). α-tubulin was used as a loading control and showed no significant changes within or between conditions following training (p>0.05).
Figure. 6.1
Total protein content of PGC-1α (a), SIRT-1 (b), p53 (c), GLUT4 (d), AKT (e) and representative blots corrected to α-tubulin (f) before (pre) and after (post) 8-weeks of control (CON; n=9), cycle ergometry (CYC; n=11) or small-sided games (SSG; n=10) conditions. Post values are expressed as fold change relative to pre values. Data reported as Mean ± SEM.
Figure 6.2
Total protein content of Mitochondrial Complex I subunit (a), Complex II subunit (b), Complex III subunit core2 (c), Complex IV subunit II (d), ATP synthase subunit-α (e) and representative blots corrected to α-tubulin (f) before (pre) and after (post) 8-weeks of control (CON; n=9), cycle ergometry (CYC; n=11) or small-sided games (SSG; n=10) conditions. Post values are expressed as fold change relative to pre values. Data reported as Mean ± SEM.
6.5: Discussion

The present study examined the efficacy of rugby-specific SSG as an initiative to improve risk-factors associated with the prevention of T2DM. The main findings revealed that 8 weeks of either SSG or CYC training induced comparable improvements in TB-FM, aerobic capacity, estimated insulin sensitivity and glucose AUC. Although training was matched for internal load and duration, SSG was the only condition to decrease insulin AUC, and to increase TB-FFM and leg strength. Furthermore, the control group showed an increase in TB-FM, IA-FM and insulin AUC. Despite these improvements in clinical risk-factors for the SSG and CYC conditions, skeletal muscle proteins associated with mitochondrial biogenesis and glucose metabolism showed no significant changes.

Common measures utilised by health professionals for the diagnosis of metabolic disease include HbA1c (%A1c), fasting glucose and insulin, estimated insulin resistance (HOMA-IR), estimated insulin sensitivity (Matsuda ISI) and/or an OGTT (Alberti et al., 2006; Matsuda & DeFronzo, 1999). Consistent with the current findings, 12 weeks of SSG (soccer) training has been reported to have no significant effect on fasting glucose concentration (Andersen et al., 2014; Randers et al., 2010a). In the present study, there were also no significant changes in HOMA-IR, which was reflected by non-significant changes in fasting glucose and insulin concentrations within all conditions. However, as all participants were normo-glycemic during the pre-intervention period, the likelihood for exercise training to improve resting glucose and insulin concentrations was reduced (Alberti et al., 2006). In contrast, both training interventions resulted in a decrease in HbA1c. This magnitude of decline in HbA1c in both groups was similar to that previously reported following ≥12 weeks of moderate-intensity endurance exercise training (Umpierre et al., 2011). The present study is the first to show that SSG training can reduce HbA1c concentration, with the decrease
similar to that occurring following traditional, continuous aerobic training. The decrease in HbA1c in both training groups should decrease the development and progression of microvascular complications (Unwin et al., 2002). For example, a 1% A1c rise above 5% A1c increases the risk of all-cause mortality and cardiovascular death by 41% (Unwin et al., 2002). These relationships indicate that both SSG and CYC conditions improved long-term glycaemic control and decreased the risk of a future cardiovascular event (Unwin et al., 2002).

The decreased HbA1c and training-induced increase in insulin sensitivity (Matsuda ISI) in both groups of the present study signifies improved glucose metabolism (Khaw et al., 2001). These results also coincided with a similar reduction for glucose AUC in both training groups. Of particular interest, SSG was the only condition to show a decrease in insulin AUC. Thus, for the first time we report that SSG is an effective training mode that can be used for improving glucose disposal and reducing insulin release in response to a standard glucose load. The results of SSG training are consistent with literature involving SIT, which has been shown to lower glucose and insulin concentrations during an OGTT (Babraj et al., 2009). Although not directly support by the current study, previous studies specific to SSG (futsal) training advocate that the social and community-based benefits of SSG may lead to better adherence and compliance within an inactive middle-aged cohort (de Sousa et al., 2014; Krustrup et al., 2010a).

The preservation of strength and muscle mass through exercise training can also help ameliorate metabolic dysfunction and prevent the onset of T2DM (Jurca et al., 2005; Krustrup et al., 2010a). In the present study, SSG training led to an increase in TB-FFM and leg strength compared to no change in either the CYC or CON groups. Previous interventions
involving SSG training (12 weeks) have reported increases in maximal isometric hamstring strength and muscle mass, when compared to continuous running, within an inactive cohort (Krstrup et al., 2010a; Krstrup et al., 2010b; Krstrup et al., 2010d). These previous findings, along with those of the present study, suggest that SSG training provides sufficient loading to induce myofibrillar protein synthesis, skeletal muscle hypertrophy and an increase in leg strength. These aforementioned adaptations in the SSG condition may also provide a mechanism for the observed decrease in insulin AUC that was not observed in CYC (Ishii et al., 1998). Importantly, the increased leg strength and TB-FFM indicates a potential advantage of SSG training over continuous, aerobic training to improve glucose metabolism and reduce risk-factors associated with T2DM (Ishii et al., 1998; Jurca et al., 2005).

Abdominal adiposity is also correlated with the development of cardiovascular and metabolic diseases, with this relationship persisting after accounting for the effects of increased TB-FM (Després & Lemieux, 2006). In the present study, TB-FM deceased by 2.6% in SSG and 2.9% in CYC. These reductions are similar to previous observations following 12 weeks of SSG (soccer) training in inactive men, which report a decrease in fat-mass by 3.0%, compared to a 1.8% decrease with continuous running (Krstrup et al., 2009). In the present study, there was also an interaction effect of IA-FM for both training conditions, compared to CON; within all groups no significant changes were evident in body mass, BMI or WHR. Regardless of the small changes in anthropometry, SSG training was equally effective as CYC at improving TB-FM and IA-FM, which are two important risk-factors associated with the development of T2DM (Després & Lemieux, 2006).

Previous research has demonstrated a greater increase in VO_{2max} with SSG (soccer, 13%) compared to continuous running (8%) (Krstrup et al., 2009). In comparison, the current
study observed similar increases in sub-maximal VO₂ between conditions (19.1% in CYC and 18.9% in rugby SSG). This increase in VO₂ coincided with an increase in test duration and workload at 80% HR_{max} (Table 6.1). These results indicate that SSG is as effective as CYC for inducing adaptations in aerobic capacity within a previously inactive population, especially when training is matched for internal training load. From a clinical perspective, men with high aerobic capacity (≥35.7 mL·kg⁻¹·min⁻¹) have been shown to be nearly two-thirds less likely to develop metabolic syndrome (Laaksonen et al., 2002). These improvements in aerobic capacity have the potential for preventing the development of metabolic syndrome and associated co-morbidities (Laaksonen et al., 2002).

Despite the positive adaptations of the aforementioned clinical risk factors within the present study, there were no significant changes in the content of skeletal muscle proteins associated with glucose regulation and mitochondrial biogenesis (Figure 6.1 and 6.2). Improvements in insulin sensitivity and glucose uptake have been shown to be facilitated by increased GLUT4 and Akt content (Andersen et al., 2014; Gonzalez & Mcgraw, 2006; Goodyear & Kahn, 1998). An important function of Akt is to mediate the metabolic actions of insulin to stimulate cellular glucose transport (Frøsig et al., 2007; Whiteman et al., 2002). Given that GLUT4 is highly abundant in skeletal muscle and is associated with enhanced glucose disposal and insulin action, there has been extensive interest in therapeutic strategies to increase Akt and GLUT4 expression in cohorts at risk of developing metabolic disorders (Frøsig et al., 2007; Whiteman et al., 2002). To our knowledge, there are no other published findings that have investigated changes in the content of proteins associated with glucose regulation in response to rugby-specific SSG training. In the present study, both SSG and CYC training were associated with favourable changes in blood chemistry relating to glucose disposal (OGTT); however, there were no corresponding increases in GLUT4 or Akt protein
content. One possible explanation is that the improved insulin sensitivity with exercise may be more dependent on increased translocation of GLUT4 to the cell surface, rather than an increase in GLUT4 abundance (Hansen, Wang, Marshall, Holloszy, & Mueckler, 1998). As we did not measure GLUT4 translocation in the present study, further research is required to test this hypothesis.

This is the first study to investigate adaptations of mitochondrial complexes I-V in response to SSG, with both training groups showing no significant effect on the protein abundance of these complexes (Figure 6.2). Both SIT and aerobic training have been associated with increases in the expression and activity of COX II and IV in healthy trained adults (Gibala et al., 2006; Little et al., 2010); however, an increase (Hood et al., 2011) or no change (Skleryk et al., 2013) has also been reported in inactive adults. As the intensity and duration of exercise has a direct effect on COX activity and expression (Dudley, Abraham, & Terjung, 1982), it appears that the accumulated training stimulus of SSG and CYC was below the threshold required to significantly increase the protein content of these mitochondrial complexes. An additional observation from the present study is that adaptations in pulmonary measures of VO₂ were not reciprocated by any observed changes in total protein content of mitochondrial complex (I-V) within skeletal muscle. Improvements in aerobic capacity are also related to improved cardiovascular function, vascular content and resistance (Krstrup et al., 2010d). Accordingly, the absence of significant changes in the protein content of the mitochondrial complexes (I-V) in the present study suggest that the predominant training adaptations were cardiovascular (Knoepfli-Lenzin et al., 2010), and/or adaptations of mitochondrial functioning/efficiency (Egan & Zierath, 2013; Little et al., 2010). However, further research is required to verify these hypotheses.
Given the absence of significant changes in the protein content of the mitochondrial complexes, it is not surprising that there were no significant changes in other proteins associated with mitochondrial biogenesis. Both SIRT1 and p53 are two of the many proteins that have been reported to acutely regulate PGC1-α, and thus may contribute to exercise-induced mitochondrial biogenesis (Bartlett et al., 2012; Gurd et al., 2010; Little et al., 2010). In the present study, there were no significant changes in SIRT1, p53 or PGC1-α protein content; in addition there were no changes in associated transcription factors (Tfam, NRF1, NRF2 and MEF2A) after either training intervention. There is limited literature available on the SIRT1 response to exercise in humans, with no reports of changes in the content of proteins associated with mitochondrial biogenesis following SSG training. A 16% increase in PGC-1α protein content has been reported following 6 weeks of interval training (Gurd et al., 2010). Interestingly, in the same study there was a 20% decrease in SIRT1 protein content, but a 31% increase in SIRT1 activity (Gurd et al., 2010). In contrast, an increase in SIRT1 (56%) content, no changes in PGC-1α content, and an increase in PGC-1α nuclear abundance (~25%) has been reported in response to SIT training (Little et al., 2010). More recently, using rat models, p53 has emerged as a potential acute regulator of mitochondrial content and function (Bartlett et al., 2012), although there are no published studies involving exercise training in humans. As such, the measurement of p53 provides an additional novel element to the current study even though there was no change of total protein content in response to either SSG or CYC training within an inactive population. Similar to the results of the present study, no significant changes in mitochondrial protein content in response to exercise training (aerobic and/or resistance, intermittent) has previously been reported in sedentary and inactive cohorts (De Filippis et al., 2008; Skleryk et al., 2013). In addition to these previous reports (De Filippis et al., 2008; Skleryk et al., 2013), results of the current study suggest that longer (>12 weeks) training programs may be required in previously
inactive men to promote increases in skeletal muscle protein content associated with mitochondrial biogenesis and glucose regulation.

Despite the potential glucose regulatory benefit of exercise, some limitations in the present study should be acknowledged. Firstly, although the number of participants was typical for muscle biopsy studies, it could be conceived as relatively low, and the effect of this on the statistical power of the muscle analyses is accepted as a limitation. Further, a sub-maximal VO₂ test was conducted and thus the ability to compare results to previous studies measuring VO₂ max is compromised. However, this is unlikely to affect the findings of the current study as sub-maximal tests are well recognised as appropriate and sensitive measures of aerobic capacity. Additionally, the use of age-predicted (220 bpm – age) HR max is not anchored against a true individual HR max, which may create differences in relative intensity between participants and the determination of sub-maximal VO₂ during the GXT, and hence should be acknowledged as such. However, it should be noted that the use of age-predicted HR max and sub-maximal VO₂ are sensitive to experimental manipulation and therefore appropriate for assessing training adaptation in aerobic capacity. Finally, the knee injury sustained as results from SSG training may be considered as a limitation with this training method and further data on longitudinal effects of such training on orthopaedic injuries should be considered.

6.6.1: Conclusions

For the first time, our results indicate that SSG training is an effective alternative to continuous cycling for improving metabolic risk-factors associated with the prevention of T2DM. The current study revealed improvements in glycaemic control, glucose AUC, aerobic capacity, abdominal and total-body fat-mass in response to both CYC and SSG training, while SSG showed additional improvements in insulin AUC, muscle mass and
lower-body strength. Despite these improvements in response to CYC and SSG training, there were no changes in the content of skeletal muscle proteins associated with glucose regulation and mitochondrial biogenesis. As such, additional research should focus on longer (>8 weeks) training programs to investigate adaptations in skeletal muscle protein content in middle-aged, inactive men. Given previous reports of greater motivation and enjoyment associated with participation in team sports, the incorporation of SSG into an exercise training approach may encourage long-term compliance for increased levels of physical activity and the overall prevention of T2DM (Krstrup et al., 2010a).
CHAPTER SEVEN

Rugby-specific small-sided games is as effective as cycle ergometry at stimulating an acute inflammatory and glucose response in middle-aged, inactive Indigenous Australians men

As accepted for publication

7.1: Abstract

**Aim**: This study investigated the acute effects of two exercise modes, including CYC and rugby-specific SSG on inflammation and glucose regulation within an Indigenous Australian population.

**Methods**: Ten inactive, untrained Indigenous male participants volunteered to participate and were not clinically diagnosed with cardiovascular or metabolic disorders. Following baseline testing and in a randomised cross-over design participants completed two exercise protocols (CYC and SSG) of 40 min duration separated by 7 d recovery. Fasting venous blood was collected pre, post, 30 min, 60 min and 240 min post-exercise for analysis of glucose, insulin, cortisol and inflammatory markers of TNF-α, IL-1β, IL-6, IL-1ra and CRP.

**Results**: IL-6 and IL-1ra were significantly (p<0.05) increased within the 240 min post-exercise period, without significant differences between protocols (p>0.05). There were no significant changes within or between protocols for TNF-α, IL-1β and CRP (p>0.05). A comparison of HOMA-IR between resting and 240 min post-exercise shows a change from a baseline value of 4.44 ±3.71 to 1.76 ±1.67 HOMA-IR in CYC (p<0.05) and to 1.54 ±1.33 HOMA-IR in SSG (p<0.05), without differences between sessions (p>0.05).

**Conclusion**: This study identified similar acute inflammatory and glucose regulatory responses between CYC and SSG. Prescribing rugby-specific SSG as a mode of physical activity may provide Indigenous populations with a community-based approach to promote increased engagement in physical activity and assist in the acute regulation of glucose disposal and inflammatory cytokines.
7.2: Introduction

In recent decades there has been a marked change in the lifestyle of many Indigenous groups around the world (Cleland & Sattar, 2005). These lifestyle changes involve cultural isolation, psychological stress, physical inactivity and the incorporation of a westernised diet (Cleland & Sattar, 2005; O’dea, 2005; Rowley et al., 1997). Such changes represent a serious health burden for Indigenous communities, evident through the increased incidence of obesity, T2DM and CVD (Cleland & Sattar, 2005; O’d ea, 2005; Zimmet, Shaw, & Alberti, 2003). Additionally, the emerging morbidity and mortality rates of T2DM and CVD have significant implications for the development of health promotion strategies that specifically target disease prevention. As such, information derived from studies targeting physical activity strategies for specific ethnic groups may provide improved preventative interventions that are culturally appropriate, relevant and evidence-based (Cleland & Sattar, 2005; Rowley et al., 1997; Rowley et al., 2003; Wang & Hoy, 2007).

Recent studies examining the health status of Indigenous Australians highlights obesity, infection and/or smoking as the main causes of elevated inflammatory biomarkers and resultant chronic disease development (Rowley et al., 2003; Wang & Hoy, 2007). Specifically, CRP is predominately up-regulated in the hepatocytes under the control of IL-6, IL-1β and TNF-α (Fischer, 2006; Pedersen & Febbraio, 2008). The prolonged presence of such inflammatory markers creates a heightened state of chronic inflammation that is regarded as a predictor and instigator of increased risk of future development of T2DM and CVD (Petersen & Pedersen, 2005).

Conversely, acute exercise-induced IL-6 release from skeletal muscle results in the subsequent secretion of IL-1ra and cortisol; stimulating an anti-inflammatory response
Chapter 7: Study 5

(Fischer, 2006). As a counter to disease development, research emphasizes primary prevention of T2DM and CVD through exercise-based health programs, particularly aimed at improving physical activity levels and reducing the inflammatory state (Brukner & Brown, 2005; Mcdermott, Rowley, Lee, Knight, & O'dea, 2000; Rowley et al., 2000a). Indeed, regular exercise training has been shown to decrease pro-inflammatory cytokines and delay the deterioration of glucose tolerance and insulin sensitivity (Eriksson & Lindgärde, 1991; Okita et al., 2004; Zimmet et al., 2003). However, despite literature supporting lifestyle and exercise interventions across different ethnic cohorts, there is no information relating to acute exercise based research within Indigenous Australians (Pan et al., 1997; Rowley et al., 2000b; Unwin et al., 2002; Zimmet et al., 2003). As such, prior to providing specific exercise training recommendations (modality, intensity and duration), further research is required to report the acute exercise-induced inflammatory and glucose homeostasis responses within this Indigenous population.

Engagement in physical activity as a preventative measure of chronic disease development contains specific ethnic bias relating to assumptions on equipment availability, facilities and social importance (Thompson & Gifford, 2000; Zimmet et al., 2003). As such, traditional gym-based exercise modes (Mendham et al., 2011; Okita et al., 2004) as a sustainable intervention to reduce disease risk may not be optimal in Indigenous communities. Given cultural and social issues involved in developing an ethnicity-specific health intervention (Zimmet et al., 2003); the modification of physical activity will be more likely to succeed if reinforced through group participation as opposed to individualised exercise and lifestyle based programs (Thompson & Gifford, 2000). Team sports such as rugby league are popular within Indigenous Australian communities and may reinforce group participation and cohesion (Andersen et al., 2010b). Thus, modified team sport, such as modified rugby in the
form of SSG may be an achievable option to reverse low physical activity levels within Indigenous populations (Thompson & Gifford, 2000). Accordingly, the current study aimed to assess the acute effects of CYC and rugby specific SSG on the biochemistry relating to inflammation and glucose homeostasis within an Indigenous Australian population. It was hypothesised that when matched for intensity modified rugby would not differ in the post-exercise inflammatory response to cycle ergometry. Further, the acute inflammatory response in both modes would be indicative of an acute increase in anti-inflammatory markers IL-6 and IL-1ra following exercise.

7.3: Methods

7.3.1: Participant recruitment
Participants volunteered from a regional Indigenous Australian community, through the support of local Indigenous members. Participants comprised of 10 sedentary males who identified as Indigenous Australian (with at least one parent of Aboriginal descent) and were non-smokers and not clinically diagnosed with CVD or metabolic disorders. The study was approved by the Research in Human Ethics Committee of Charles Sturt University. Prior to testing procedures, all participants were familiarised with all testing procedures, provided verbal and written consent and completed a pre-exercise health questionnaire.

7.3.2: Overview
Testing procedures started at standardised times from 0730 to 0900 h, following an overnight fast (10-12 h). On two separate occasions, participants completed two respective exercise protocols (CYC and SSG) in a randomised cross-over design, each separated by 7 d recovery. Participant’s physical activity and diet were standardised and recorded 24 h prior to testing.
and then replicated throughout the remaining testing procedures. During each protocol and 240 min after all testing sessions, participants remained fasted and consumed water *ab libitum* (~500 mL).

### 7.3.3: Baseline testing

On arrival at baseline testing, measures of height (stadiometer: Custom CSU, Bathurst, Australia), body mass on calibrated scales (HW 150 K; A&D, Bradford, MA, USA) and waist (measured just above the iliac crest) and hip girths (greatest posterior protuberance of the buttocks) (steel tape, EC P3 metric graduation, Sydney, Australia) were obtained (Hill et al. 2007). Manual blood pressure was obtained with an aneroid sphygmomanometer and cuff (Welch-Allyn, Arden, CA, USA) expressed as the mean of three measurements after being seated for 5 min. A supine whole body DXA scan (XR800, Norland, Cooper Surgical Company, Trumbull, CT, USA) was conducted with scanning resolution set at 6.5 x 13.0 mm, and scanning speed was set at 130 mm·s⁻¹. Whole body scans were analysed (Illuminatus DXA, ver.4.2.0, Trumbull, CT, USA) for TB-FM (Kim et al., 2002).

Measures of aerobic capacity were obtained via a GXT to determine sub-maximal VO₂. Pulmonary gas exchange was measured by determining O₂ and CO₂ concentrations and ventilation to calculate VO₂ consumption using a metabolic gas analysis system (ParvoMedics, True2400, East Sandy, UT, USA). Prior to each session, the flow meter was calibrated using a three litre syringe, while gas analysers were calibrated for fractional gas concentration with a gravimetric gas mixture of known concentrations (CO₂, 4.1 ±0.1 %; O₂, 15.7 ±0.2 %), in accordance with the manufacturer’s instructions. The GXT was performed on an electronically-braked cycle ergometer (LODE Excalibur Sport, LODE BV, Groningen, The Netherlands), which started at 25 W and increased by 25 W every min. Heart rate
(Vantage NV, Polar, Kempele, Finland) was recorded each min throughout the protocol, and subjects exercised until attainment of 80% age-predicted (220-age) HR\textsubscript{max}. VO\textsubscript{2} was measured continuously throughout the exercise protocol and reported as a VO\textsubscript{2} at 80% HR\textsubscript{max}.

7.3.4: Rugby specific small-sided games

The protocol consisted of interval sessions, with participants completing 40 min of six-a-side on a small pitch (width: 40 m; length: 60 m). The modified rugby session was played under touch football rules. The game required each team six ‘plays’ whilst in possession of the ball; each play requiring players to pass the ball backwards to an ‘on side’ team member. Defending players were required to touch their opponent with one hand. Following a successful touch, game play would restart with a ‘play the ball’, at this time requiring the line of defending players to be 5 m from the position of each ‘play the ball’ (Kennett et al. 2011). The session comprised of 4 x 10 min bouts, interspersed by 2 min passive recoveries. A Global Positioning Satellite (GPS) device (SPIetite, GPSports, Canberra, Australia) was worn in a customised harness between the scapulae to quantify distance and mean speed (m min\(^{-1}\)) of movement patterns during the session (Coutts & Duffield, 2010). At the end of each 10 min period, HR and RPE (Borg’s 6-20 scale) were recorded. Additionally, 30 min post-exercise exercise perception was recorded using the RPE scale (Hill-Haas et al., 2011) and rating the question; how challenging did you find the exercise session? on a scale of 1-10 (1 = Not at all, 10 = Very much). Using the intrinsic motivation inventory scale participants completed a question from the interest/enjoyment subscale the exercises are fun to do, ranging on a scale of 1-7 (1 = not at all true, 4 = somewhat true, 7 = very true) (Mcauley, 1989).
7.3.5: Cycle ergometry

The CYC session was conducted on Monark stationary cycle ergometers (Monark 828E, Monark Exercise AB, Varburg, Sweden) and comprised of 4 x 10 min bouts, at a target intensity of 80-85% HR\textsubscript{max}, interspersed by 2 min passive recoveries. During the session, cadence was maintained at 60-65 rpm and individual resistance adjusted to maintain target HR zones. Given the different exercise modes conditions it is recognised the inherent difficulties of matching external load or metabolic cost. However, despite this limitation, in an attempt to best match the intensity between conditions the respective conditions were designed to elicit similar responses. At the end of each 10 min interval HR and RPE were recorded, including session-RPE 30 min following exercise. The intensity and duration of the CYC condition was design to match the SSG condition and approximate mean target HR zone of 80–85% HR\textsubscript{max}.

7.3.6: Venous blood sampling and analysis

Blood was collected during baseline testing for analysis of fasting total cholesterol (Enzymatic Method and Polychromatic Endpoint Technique), HDL (Accelerator Selective Detergent Methodology), LDL (Friedwald Equation), triglycerides (Enzymatic Method and Biochromatic Endpoint Technique: Dimension Xpand Plus, Siemens Healthcare Diagnostics, Sydney, Australia), total leukocyte count (Cell Counter: Cell-Dyn 3200, Abbott Laboratories, Abbott Park, IL, USA) and HbA1c (High-Performance Liquid Chromatography: Bio-Rad Variant, Bio-Rad Laboratories, Sydney, Australia). During the respective protocols, 20 mL was collected at each time point for analysis of glucose, lactate (ABL825 Flex Analyser, Radiometer Medical ApS, Bronshoj, Denmark), insulin, cortisol (Solid-phase Chemiluminescent Enzyme Immunometric Assay: Immulite 2000, Siemens Healthcare Diagnostics, Los Angeles, CA, USA) and CRP (Particle Enhanced Turbidimetric Immunoassay: Dimension Xpand Plus, Siemens Healthcare Diagnostics, Sydney, Australia).
Analysis of biochemistry variables glucose, lactate, insulin, cortisol and CRP showed intra and inter-assay coefficients of variation between 4.0 - 7.4 %. IL-6, IL-1β, IL-1ra and TNF-α were measured at each time point using a monoclonal antibody, specific to the cytokine pre-coated onto the microplate (Sandwich Enzyme Immunoassay - ELISA: Quantikine, R & D Systems, Minneapolis, MN, USA), with intra and inter-assay coefficients of variation between 4.3 – 5.6 %. HOMA-IR was calculated using the formula, (fasting insulin x fasting glucose)/22.5 (Matthews et al. 1985; Wallace et al. 2004). Serum or plasma was collected following centrifugation at 3500 rpm for 15 min at 4°C. Aliquots were frozen at -80°C and -20°C for EDTA and SST, respectively. For analysis of glucose, leukocytes and HbA1c, whole blood was refrigerated (4°C) until further analysis.

7.3.7: Statistical Analyses

All data are reported as mean ±SEM. Within and between protocol and blood measure time-point differences were assessed using a two-way repeated measures ANOVA (condition x time). When significant differences were observed, Tukey’s pairwise comparisons were employed to assess the source of significance that was set at p<0.05. All statistical analyses were performed using PASW™ for MS-Windows v17.0 (Statistical Package for the Social Sciences, Chicago, IL, USA).

7.4: Results

All resting and descriptive measures of anthropometry, DXA (TB-FM %), blood pressure, sub-maximal VO₂ (at 80% HRmax) and resting venous blood values are presented in Table 7.1. Participants showed high levels of adiposity, CRP concentrations and insulin resistance, as evident through elevated fasting insulin concentrations and HOMA-IR.
Table 7.1

Baseline characteristics (n=10). Data provided as mean ± SEM

<table>
<thead>
<tr>
<th></th>
<th>Resting Values</th>
<th>Desirable Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>38.5 ± 3.2</td>
<td>-</td>
</tr>
<tr>
<td>Sub-Maximal oxygen consumption (mL kg⁻¹ min⁻¹)</td>
<td>30.8 ± 1.7</td>
<td>-</td>
</tr>
<tr>
<td>Body mass index (kg m²)</td>
<td>31.9 ± 2.0</td>
<td>&lt; 25</td>
</tr>
<tr>
<td>Systole Blood Pressure (mmHg)</td>
<td>131 ± 2</td>
<td>&lt;130</td>
</tr>
<tr>
<td>Diastole Blood Pressure (mmHg)</td>
<td>84 ± 2</td>
<td>&lt;90</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>103.6 ± 5.9</td>
<td>&lt;102</td>
</tr>
<tr>
<td>Waist to Hip Ratio</td>
<td>0.95 ± 0.03</td>
<td>&lt; 0.90</td>
</tr>
<tr>
<td>Total Body – Fat Mass (%)</td>
<td>27.8 ± 3.6</td>
<td>&lt; 25</td>
</tr>
<tr>
<td>Total Cholesterol (mmol L⁻¹)</td>
<td>5.10 ± 0.28</td>
<td>&lt; 5.5</td>
</tr>
<tr>
<td>High Density Lipoprotein (mmol L⁻¹)</td>
<td>1.13 ± 0.09</td>
<td>&gt; 1.0</td>
</tr>
<tr>
<td>Triglycerides (mmol L⁻¹)</td>
<td>1.55 ± 0.23</td>
<td>&lt; 2.0</td>
</tr>
<tr>
<td>Cholesterol Hazard Ratio</td>
<td>4.78 ± 0.45</td>
<td>&lt; 4.5</td>
</tr>
<tr>
<td>HbA1c (%A1c)</td>
<td>5.69 ± 0.19</td>
<td>&lt; 7</td>
</tr>
<tr>
<td>Glucose (mmol L⁻¹)</td>
<td>5.38 ± 0.21</td>
<td>&lt; 5.5</td>
</tr>
<tr>
<td>Insulin (µIU mL⁻¹)</td>
<td>17.7 ± 4.59</td>
<td>5-15</td>
</tr>
<tr>
<td>Insulin resistance (HOMA)</td>
<td>4.44 ± 1.17</td>
<td>&lt; 4</td>
</tr>
<tr>
<td>Total Leukocyte Count (10⁹ L⁻¹)</td>
<td>6.85 ± 0.59</td>
<td>4-10</td>
</tr>
<tr>
<td>CRP (mg L⁻¹)</td>
<td>3.05 ± 0.65</td>
<td>&lt; 2.9</td>
</tr>
<tr>
<td>IL-6 (pg mL⁻¹)</td>
<td>0.96 ± 0.22</td>
<td>&lt; 1.04</td>
</tr>
<tr>
<td>TNF-α (pg mL⁻¹)</td>
<td>2.66 ± 0.44</td>
<td>&lt; 4.17</td>
</tr>
</tbody>
</table>
7.4.1: Small-sided games and cycle ergometry demands

Total distance covered during the CYC was 2696 ± 398 m, at 67 ± 10 m min⁻¹, involving 140 ± 78 m of higher-speed running above 14 km h⁻¹. The mean HR responses for the SSG and CYC protocols were 83.1 ± 6.6% and 81.4 ± 4.8% HR_max, respectively, with no significant difference between the respective protocols (p > 0.05). Additionally, session RPE was not significantly different between protocols at 14.1 ± 1.7 AU for SSG and 13.9 ± 1.9 AU for CYC (p > 0.05). For the question; how challenging did you find the exercise session? Participants rated SSG at 6.6 ± 2.0 and CYC at 7.4 ± 1.8 AU with no significant differences between protocols (p > 0.05). For the question; the exercises are fun to do, results showed a significantly higher rating (p < 0.05) at 6.6 ± 0.5 for SSG to 5.2 ± 1.3 in CYC.

7.4.2: Inflammatory response to acute exercise

The acute post-exercise response within the SSG and CYC protocols for IL-6 IL-1ra, TNF-α and IL-1β are shown in Figure 7.1. Between protocol comparisons indicated no significant differences at any time point within the respective inflammatory responses (p > 0.05). Specifically, within both protocols IL-6 increased immediately post exercise, whilst IL-1ra increased progressively throughout the 240 min period post exercise (p < 0.05). Additionally, CRP, TNF-α, and IL-1β showed no significant post-exercise changes between or within both protocols (p > 0.05).
Figure 7.1

Data reported as Mean ± SEM. Response of IL-6, IL-1ra, TNF-α, and IL-1β within and between the respective protocols.

Significant change within the CYC protocol \(^a\) \(p<0.05\); Significant change within the SSG protocol \(^b\) \(p<0.05\); 240 min post values
7.4.3: Cortisol, insulin, glucose and lactate response to acute exercise

The acute post-exercise response in SSG and CYC protocols for cortisol, fasting glucose, and fasting insulin and lactate are represented in Figure 7.2. No significant differences were evident between protocols for cortisol (p>0.05). Cortisol showed no significant increases immediately post exercise (p>0.05) and progressively decreased below resting values at 240 min post-exercise. Between protocol comparisons for fasting glucose showed a significantly higher post-exercise peak in SSG (p<0.05). Within the CYC protocol, fasting glucose values showed a progressive decline below baseline until 240 min post-exercise, whilst SSG increased immediately post-exercise (p<0.05). Additionally, fasting insulin showed a significant increase immediately post-exercise (p<0.05). Conversely, a lower response was evident immediately post-exercise in the CYC protocol (p<0.05). A comparison of HOMA-IR between resting and 240 min post-exercise showed a change from 4.50 ±3.68 to 1.76 ±1.67 AU in the CYC protocol (p<0.05) and 4.37 ±3.8 to 1.54 ±1.33 AU in the SSG protocol (p<0.05), without differences between protocols (p>0.05). The post-exercise increase in blood lactate showed no differences between the SSG and CYC protocols (p>0.05).
Figure 7.2
Data reported as Mean ± SEM. Response of cortisol, glucose, insulin and lactate within and between the respective protocols.
Significant change within the CYC $^a$ p<0.05; Significant change within the SSG $^b$ p<0.05; 240 min post values significantly different to pre values in both protocols $^c$ p<0.01; Significant difference between protocols $^d$ p<0.05.
7.5: Discussion

The acute inflammatory and glucose responses to cycle ergometry and modified rugby within a sedentary, male Indigenous Australian population were investigated to assess the efficacy of SSG as an alternative exercise intervention for this community. The group examined showed increased insulin resistance (HOMA-IR and fasting insulin values), and above the desirable range of adiposity (TB-FM %), waist to hip ratio, cholesterol hazard ratio, and CRP values. The present results showed modified rugby as SSG to have similar acute inflammatory and glucose responses to a traditionally prescribed exercise modality (i.e. CYC). Given the respective conditions were prescribed to be similar in duration (40 min) and intensity (RPE, HR, blood lactate) (Hill-Haas et al., 2011), the current results suggest similar effectiveness in inducing acute inflammation and glucose control.

Acute exercise improves glucose homeostasis, insulin sensitivity and anti-inflammatory responses of skeletal muscle and blood based markers within Caucasian populations (Fischer, 2006; Kindermann et al., 1982; Kramer & Goodyear, 2007; Mendham et al., 2011; Petersen & Pedersen, 2005). In agreement, the present study showed that within an Indigenous population, both protocols increased IL-6 post-exercise, followed by an increase in IL-1ra throughout the 240 min post-exercise period (Mendham et al., 2011; Pedersen & Febbraio, 2008; Petersen & Pedersen, 2005). These observations suggest that the post-exercise release of IL-6 and subsequent secretion of IL-1ra represent an exercise-induced anti-inflammatory response (Fischer, 2006; Kramer & Goodyear, 2007). Accordingly, exercise may stimulate positive acute inflammatory responses, potentially providing a therapeutic avenue to treat and/or inhibit insulin resistance if applied over a chronic training program within an Indigenous Australian population (Pedersen et al., 2003).
Conversely, CRP and the pro-inflammatory cytokines TNF-α and IL-1β showed no exercise-induced changes. These data fit the suggested response that contraction-induced release of IL-6 promotes an anti-inflammatory environment by stimulating the production of IL-1ra and inhibiting a pro-inflammatory response (TNF-α and IL-1β) following exercise (Pedersen & Febbraio, 2008; Petersen & Pedersen, 2005). Furthermore, CRP is reported to peak within the 24-48 h post-exercise, which may explain the blunted CRP response observed in the current study (Pedersen & Febbraio, 2008). Within this limitation, the present findings suggest that when matched for similar intensities and duration, the prescription of SSG within an Indigenous population induces similar acute inflammatory temporal responses to those reported within Caucasian groups involving gym and laboratory research (Pedersen & Febbraio, 2008). Consequently, isolated exercise may elicit acute anti-inflammatory effects (Kramer & Goodyear, 2007; Thompson et al., 2001), and if encountered repeatedly (i.e. regular training), may be an important foundation for prevention of the chronic systemic inflammation and/or ensuing disease state (Albert, 2007; Pedersen & Febbraio, 2008; Valery et al., 2009).

Insulin concentrations following exercise depend on catecholamine and glucose responses (Kindermann et al., 1982). This study showed that glucose concentration decreased, causing a delayed insulin response within the CYC protocol immediately post-exercise. Conversely, the SSG protocol increased blood glucose concentration, which is reported to override the suppressive effect of catecholamine’s and cause a subsequent increase in insulin (Kindermann et al., 1982). Accordingly, the differences in post-exercise insulin response between protocols are potentially due to the different glucose responses within each respective protocol; likely a result of engagement in transient but higher-intensity efforts during SSG (Kindermann et al., 1982; Krustrup et al., 2010a). Despite the immediate
difference in glucose regulatory responses, both protocols showed similar trends from 30 to 240 min post-exercise. Accordingly, this study showed that HOMA-IR pre-exercise decreased to within a more desirable range by 240 min post-exercise for both SSG and CYC (Jamurtas et al., 2006). As such, the acute contraction of skeletal muscle is reported to directly improve glucose metabolism, and modify cytokine production (Kramer & Goodyear, 2007; Pedersen & Febbraio, 2008; Thompson et al., 2001).

Recreational football (soccer) has previously been shown to stimulate training adaptations through alterations in musculoskeletal, metabolic and cardiovascular health within Caucasian populations (Andersen et al., 2010b; Hill-Haas et al., 2011; Krstrup et al., 2010a). The similarities in acute responses between modes in the present study suggest that with chronic application, both protocols may provide some health benefits via a decreased inflammatory state and improved glucose disposal (Fischer, 2006; Pedersen & Febbraio, 2008), from a cultural perspective, SSG provides exercise through group and community involvement, which may create a more palatable option for Indigenous communities (Rowley et al., 2000a; Thompson & Gifford, 2000). Furthermore, despite the similar intensity, SSG was rated more ‘fun to do’ in comparison to CYC, which may assist with compliance when applied as a chronic training program (Andersen et al., 2010b). Given the increasing rates of obesity, T2DM and CVD risk factors evident in the Indigenous Australian population (Medermott et al., 2000), such programs may be more effective to improve health outcomes. However, despite such assumptions, no training programs with a focus on inflammatory or glucose regulatory markers have been applied within Indigenous Australian populations, and future research is required to assess such physiological responses and compliance involving the respective exercise modalities as a potential avenue for disease prevention.
7.5.1: Conclusions

In summary, the present study identified similar acute inflammatory and glucose regulatory responses between CYC and SSG modes in an Indigenous Australian population. Consequently, both exercise modalities may be appropriate to obtain acute responses promoting an increased health benefits involving inflammation and glucose homeostasis. Specifically, the encouragement of SSG may provide Indigenous populations with a more community based physical activity intervention as opposed to individualised exercise sessions such as laboratory or gym-based CYC.
A 12-week sports-based exercise program for inactive Indigenous Australian men improved clinical risk factors associated with type 2 diabetes

As accepted for publication

8.1: Abstract

Aim: This study assessed the impact of a 12-week sports-based exercise intervention on glucose regulation, anthropometry and inflammatory markers associated with the prevalence of type 2 diabetes mellitus (T2DM) in Indigenous Australian men.

Methods: Twenty-six inactive Indigenous Australian men (48.6±6.6 y) were randomised into an exercise (n=16) or control (n=10) conditions. Training included ~2-3 days/week for 12 weeks of sports and gym exercises in a group environment, whilst control participants maintained normal activity and dietary patterns. Pre- and post-intervention testing included: anthropometry, peak aerobic capacity, fasting blood chemistry of inflammatory cytokines, adiponectin, leptin, cholesterol, glucose, insulin and C-peptide. An oral glucose tolerance test measured glucose, insulin and C-peptide 30, 60, 90 and 120 min post 75 g glucose ingestion.

Results: The exercise condition decreased insulin area under the curve (25±22%), increased estimated insulin sensitivity (35±62%) and decreased insulin resistance (9±35%; p<0.05), compared with control (p>0.05). The exercise condition decreased in body mass index, waist circumference and waist to hip ratio (p<0.05), compared to control (p>0.05). Leptin decreased in the exercise group, with no changes for adiponectin (p>0.05) or inflammatory markers (p>0.05) in either condition. Aerobic capacity variables showed significant increases in peak oxygen consumption for the exercise condition compared to no change in control (p>0.05).

Conclusion: Findings indicate positive clinical outcomes in metabolic, anthropometric and aerobic capacity variables. This study provides evidence for sport and group-based activities leading to improved clinical risk factors associated with T2DM development in clinically obese Indigenous Australian men.
8.2: Introduction

An estimated 75% of Indigenous people living in non-remote areas report sedentary behaviour and low levels of physical activity (Australian Institute of Health and Welfare, 2011). In turn, physical inactivity is reported to promote the development of obesity and is strongly associated with preventable chronic diseases such as T2DM and CVD (Alberti et al., 2006; Kahn et al., 2006). Of note, both disease states are disproportionately high in the Indigenous Australian population (Alberti et al., 2006; Australian Institute of Health and Welfare, 2011; Brown, 2012). Increasing the levels of physical activity within high-risk Indigenous communities may assist in preventing the development of chronic diseases. Accordingly, given the prevalence for lifestyle-related chronic diseases in Indigenous populations, the need for evidence-based strategies to reduce physical inactivity and associated risk of non-communicable disease is essential (Neesham & Garnham, 2012). However, to date there are very few published reports on exercise training as a primary prevention strategy for metabolic and cardiovascular disease within Indigenous people.

Of particular focus, glucose regulatory, (O'dea & Rowley, 2002) chronic systemic inflammatory (Albert, 2007; Miller & Cappuccio, 2007) and anthropometric (Maple-Brown et al., 2013) indices are important risk-factors for metabolic disease and their interrelated effects on insulin resistance and atherosclerosis (Miller & Cappuccio, 2007). Specifically, training studies implemented within a range of Indigenous peoples report ameliorating metabolic disease through reductions in HbA1c, insulin action, body composition, blood lipids and blood pressure (Sukala, Page, & Cheema, 2012). However, minimal exercise interventions are specific to the Indigenous Australian population (Canuto et al., 2012; Rowley et al., 2000a), with none previously reported in Indigenous Australian men relating to glucose regulatory, inflammatory and anthropometric variables.
Regardless of ancestry, sports-specific exercise training (Krustrup et al., 2010a) or gym-based cardiovascular and resistance exercises (Canuto et al., 2012) have been successful in improving glucose regulation, inflammatory and anthropometric outcomes (Canuto et al., 2012; Krustrup et al., 2010a). Evidence-based training programs may provide effective and sustainable opportunities to improve risk-factors associated with disease development in Indigenous Australian men. Moreover, based on the community and family-orientated culture embedded within Indigenous Australian communities (Thompson et al., 2000) group and sports-specific training may be an effective approach for increasing physical activity and improving clinical risk-factors associated with T2DM (Biddle et al., 2011; Canuto et al., 2012). The geographical location of these communities dictates exposure to certain sports and thus drives specific interests. As such, the Indigenous Australian community within the present study communicated their group interest in football (rugby union and rugby league) and boxing. Accordingly, the current study was specifically structured around these interests to ensure compliance and participation within the program. The current study aimed to assess changes in clinical risk-factors following a 12-week exercise program. These include the assessment of primary glucose regulatory measures from OGTT and secondary measures of inflammatory, anthropometric and aerobic capacity variables. It was hypothesised that a sports-specific exercise intervention will assist in improving these clinical risk-factors associated with the development of T2DM within Indigenous Australian men.

### 8.3: Methods

#### 8.3.1: Participant recruitment

Over a 4 month period participants volunteered from a regional New South Wales community through the support and guidance of the local Aboriginal Medical Centre and
Men’s group. Thirty-three men of Australian Indigenous ancestry (self-identified as Indigenous Australian, with at least one parent of Aboriginal descent) were recruited and randomly (block randomisation in groups of 4) assigned by the chief investigator to an exercise (n=17) or CON (n=16) condition for pre-intervention testing. The extra participant was assigned to the exercise intervention based on anticipated drop-out and compliance rates (Canuto et al., 2012). Participant recruitment ensured a sample population representative of an inactive lifestyle (no regular planned or incidental activity of >60 min wk⁻¹) and not diagnosed with pre-existing CVD or metabolic disorders. An OGTT (75 g) at pre-intervention showed results indicative of diabetes for 6 participants, who were subsequently excluded from the study. Final sample size at post-intervention was 11 in exercise and 10 in control conditions (schematic overview of participant numbers shown in Figure 8.1). Prior to participation, Institutional Human Ethics clearance was obtained and participants provided verbal and written consent for all testing procedures.
Figure 8.1
Schematic overview of participant numbers for allocation, pre-intervention testing to post-intervention testing.
8.3.2: Baseline testing

Participants attended two pre-intervention and two post-intervention testing sessions (Figure 8.1). The first testing session comprised of a PAR-Q, anthropometric measurements, blood pressure and an OGTT. The second testing session comprised of a GXT. Anthropometric measures included stature, body mass, WC and hip circumference using standard techniques (Marfell-Jones et al., 2012). Manual blood pressure was obtained with an aneroid sphygmomanometer and cuff (Welch-Alyn, Arden, North Carolina, USA) expressed as the mean of 3 measurements after the participant had been seated for 5 min.

Participants presented to the laboratory between 0600 and 0900 h following an overnight fast (10-12 h) and remained rested for a 2 h OGTT. Participants were cannulated for the collection of venous blood samples at fasting, 30, 60, 90 and 120 min post-glucose ingestion that was standardised for all participants at 75 g of glucose diluted in 300 mL of water, ingested within a 5 min period (Fronine Lomb’s Scientific, Sydney, Australia).

A GXT determined VO$_{2peak}$ and maximal aerobic workload ($W_{max}$) and was performed on an electronically braked cycle ergometer (LODE Excalibur Sport, LODE BV, Groningen, The Netherlands). Prior to each test the metabolic gas analysis system (Parvo Medics, True2400, East Sandy, UT, USA) was calibrated. The test commenced at 25 W and increased by 25 W every min. Heart rate (Vantage NV, Polar, Kempele, Finland) was recorded each min with participants exercising until age predicted (220 - age) $HR_{max}$ or volitional exhaustion prior to attainment of $HR_{max}$. Technicians were not blinded to group allocation and did not provide encouragement to the participants during pre and post-intervention testing.
8.3.3: Exercise training condition

Total exercise duration over the 12-weeks of training was maintained at 45 and 60 min for respective types of sessions (including 5-10 min of dynamic warm-up), with exercise intensity prescribed to maintain 70-85% $\text{HR}_{\text{max}}$. Training frequency progressed from an allocated 2 sessions (weeks 1-6) to 3 sessions per week (weeks 7-12). Heart rate (Vantage NV, Polar, Kempele, Finland) was recorded during all sessions at 5 min intervals for the calculation of mean HR, and a session-RPE (Borg’s 6-20 scale) was obtained at the conclusion to calculate training load (Herman et al., 2006). All participants were provided with positive reinforcement and transportation (if required) to all data collection and training sessions.

Supervised group-based cardiovascular and resistance exercises were performed at a local fitness centre (Weeks 1-12). Specifically, these sessions (45 min) altered between strength training (free weights i.e. chest press, squats, and lunges), core exercises (sit-ups with incorporation of medicine balls) and cardiovascular training of continuous cycle ergometry, running and rowing ergometry. Resistance and/or speed (i.e. rpm or km$h^{-1}$) for individual participants was altered to maintain 70-85% $\text{HR}_{\text{max}}$. An additional session (60 min) comprised of boxing specific circuit training, including multiple stations of sparring, technique work using pads, speed ball, boxing bag, skipping, running and passive recovery. Throughout the program work to rest ratio progressed from 1:1 (weeks 1-3), 2:1 (weeks 4-6), 3:1 (weeks 7-9) and 4:1 (weeks 10-12).

The final weeks included a third weekly session of SSG (Weeks 7-12). All SSG training was conducted at an indoor multi-sports centre. Games included football (touch rugby, futsal), basketball and netball. Training duration consisted of 4 quarters, with 2-min passive recovery
and court size of 15 x 28 m. The duration of each quarter progressed from 8 min (weeks 7-9) to 10 min (weeks 10-12). Depending on participant availability player numbers altered between 6 v 6) or 7 v 7.

8.2.4: Control condition

The CON condition completed pre and post-intervention testing sessions and were required to continue their usual inactive lifestyle (no regular planned or incidental activity of >60 min per week) and nutritional patterns for 12-weeks. Participants received both verbal and written instructions expressing the importance of maintaining these patterns. After the completion of the study, the CON condition was provided with assistance to increase levels of physical activity.

8.3.5: Venous blood sampling and analyses

Fasting venous samples were collected for analysis of lipid profile, CRP, insulin, glucose, C-peptide, HbA1c, total leukocyte count, IL-6, IL-1ra, IL-1β and TNF-α. Venous blood from the OGTT was analysed for insulin, glucose and C-peptide. Following the clotting of the sample (SST) or immediately following collection (EDTA, FO), samples were centrifuged at 3500 rpm for 15 min at 4°C. Aliquots were frozen immediately at -80°C and -20°C for EDTA and SST, respectively. Whole blood was refrigerated (4°C) for a maximum of 6 h until analysis of total leukocyte count and HbA1c.

Fasting samples pre and post intervention were analysed for total cholesterol (Enzymatic method and polychromatic endpoint technique), HDL (Accelerator selective detergent methodology), LDL (Friedwald Equation), triglycerides (Enzymatic method and biochromatic endpoint technique; Dimension Xpand Plus, Siemens Healthcare Diagnostics,
Sydney, Australia), total leukocyte count (Cell counter: Cell-Dyn 3200, Abbott Laboratories, Abbott Park, IL, USA) and HbA1c (High-performance liquid chromatography: Bio-Rad Variant, Bio-Rad Laboratories, Sydney, Australia). CRP (Particle enhanced turbidimetric immunoassay: Dimension Xpand Plus, Siemens Healthcare Diagnostics, Sydney, Australia), IL-6, IL-1β, IL-1ra and TNF-α were measured (Immunoassay ELISA: Quantikine, R & D Systems, Minneapolis, MN, USA), with intra and inter-assay coefficients of variation between 2.9-4.9%. Glucose (ABL825 Flex Analyser, Radiometer Medical ApS, Bronshoj, Denmark), C-peptide and insulin (Solid-phase chemiluminescent enzyme immunometric assay: Immulite 2000, Siemens Healthcare Diagnostics, Los Angeles, CA, USA) showed intra and inter-assay coefficients of variation between 2.2-5.1%. HOMA-IR was calculated based on (fasting insulin x fasting glucose)/22.5 (Matthews et al., 1985). Area under the curve (AUC) was calculated using trapezoidal method (Allison et al., 1995). The Matsuda index was calculated as an alternative measure to whole body insulin sensitivity (Matsuda & Defronzo, 1999).

8.3.6: Statistical analyses
All data are reported as mean ± SEM. A one-way repeated measure ANOVA (pre-post intervention) was used to compare the effects of each intervention for all measured variables. A two-way repeated measures ANOVA (pre-post intervention x 5 time points of glucose load) was used to assess the effect of each intervention on glucose, insulin and C-peptide. Post-hoc paired sample t-tests were used to determine where any difference lay pre- to post-intervention within each condition. Significance was accepted at p<0.05. All data not normally distributed were log transformed prior to analysis (variables included, all inflammatory cytokines, C-peptide, HbA1c, BMI and WHR). All statistical analyses were performed using PASW™ for MS-Windows version 20.0 (Statistical Package for the Social Sciences, Chicago, IL, USA).
8.4: Results

Mean training intensity (0-12 weeks) was 82.3 ±1.6% of age-predicted HR$_{max}$ and session-RPE of 14.2 ±4.3 AU. Attendance throughout the training study was 73 ±17% (weeks 1-6) and 65 ±16% (weeks 7-12), with a mean attendance rate of 69 ±16% (0-12 weeks).

8.4.1: Anthropometry and aerobic capacity

Participant characteristics, anthropometry and VO$_{2peak}$ pre and post 12 weeks of training are provided in Table 8.1. A significant decrease within the exercise condition was evident in BMI (p=0.001), WC (p=0.015) and WHR (p=0.018), with no significant differences in the control condition (p>0.05). A significantly greater change was evident following the exercise program for body mass (p=0.042), BMI (p=0.013), WC (p=0.004) and WHR (p=0.041) compared to control. Further, a significant increase in GXT duration (17.4 ±7.8%; p=0.001) and W$_{max}$ (14.2 ±6.5%; p=0.001) was evident within the exercise condition compared to no change within the control condition (p>0.05). The pre to post change was significantly different between conditions for VO$_{2peak}$ (p=0.021), GXT duration (p=0.002) and W$_{max}$ (p=0.007).
### Table 8.1
Participant characteristics, anthropometry and peak oxygen uptake pre and post 12 weeks of exercise training and control conditions.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Exercise group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td><strong>Age (y)</strong></td>
<td>39.5 ± 3.2</td>
<td>-</td>
</tr>
<tr>
<td><strong>Height (cm)</strong></td>
<td>173.6 ± 1.4</td>
<td>-</td>
</tr>
<tr>
<td><strong>Mass (kg)</strong></td>
<td>95.4 ± 3.5</td>
<td>94.1 ± 3.6 b</td>
</tr>
<tr>
<td><strong>BMI (kg m$^2$)</strong></td>
<td>31.6 ± 0.9</td>
<td>27.1 ± 0.9 a, b</td>
</tr>
<tr>
<td><strong>Waist circumference (cm)</strong></td>
<td>103.5 ± 2.6</td>
<td>100.2 ± 2.7 a, b</td>
</tr>
<tr>
<td><strong>Hip circumference (cm)</strong></td>
<td>107.5 ± 1.6</td>
<td>106.9 ± 1.8</td>
</tr>
<tr>
<td><strong>WHR</strong></td>
<td>0.96 ± 0.01</td>
<td>0.94 ± 0.02 a, b</td>
</tr>
<tr>
<td><strong>Systolic blood pressure (mmHg)</strong></td>
<td>123.7 ± 2.5</td>
<td>123.4 ± 2.5</td>
</tr>
<tr>
<td><strong>Diastolic blood pressure (mmHg)</strong></td>
<td>79.9 ± 2.1</td>
<td>78.4 ± 2.6</td>
</tr>
</tbody>
</table>

**Graded Exercise Test**

| VO$_2$peak (mL kg$^{-1}$·min$^{-1}$) | 31.0 ± 2.6 | 34.7 ± 3.2 b | 32.1 ± 2.1 | 31.6 ± 2.0 |
| W$_{max}$ (Watts)                   | 239.3 ± 21.0 | 271.4 ± 19.4 a, b | 253.3 ± 15.9 | 240.6 ± 18.3 |
| Duration (min)                      | 8.8 ± 0.8 | 10.3 ± 0.7 a, b | 9.3 ± 0.7 | 8.8 ± 0.8 |

Data reported for pre and post variables are based on final participants numbers used for statistical analysis: Age, anthropometry and blood pressure (Exercise group n=11; Control group n=10) and peak oxygen uptake, W$_{max}$ and duration (Exercise group n=7; control group n=10).

BMI = Body mass index; WHR = Waist to hip ratio; VO$_2$peak = Peak oxygen uptake; W$_{max}$ = Maximal workload

a Significant pre to post change within condition (p<0.05);  b Pre to post change significantly different between conditions (p<0.05).
8.4.2: Blood chemistry: Inflammation and glucose regulation

Fasting blood chemistry, insulin sensitivity/resistance and inflammatory cytokines before and after training are provided in Table 8.2. Insulin AUC significantly decreased by 25 ±22% within the exercise condition (p=0.018), compared to no change within the control condition (p=0.702). The pre to post change in insulin AUC was significantly greater in the exercise compared to control condition (p=0.014). Matsuda ISI showed a significant increase of 35 ±62% within the exercise group (p=0.002), compared to a 14 ±16% decrease within the control group (p=0.041). The pre to post change in Matsuda ISI was significantly greater in the exercise compared to control condition (p=0.013). Leptin significantly decreased in the exercise conditions (p=0.048), without changes in the control condition (p=0.674). The pre to post change in leptin was significantly greater in the exercise compared to control condition (p=0.041). Adiponectin and all inflammatory cytokines showed no significant changes within or between conditions (p>0.05).
### Table 8.2
Fasting blood chemistry, estimated insulin sensitivity/resistance and inflammatory cytokines pre and post 12-weeks of training.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Exercise group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Glucose (mmol·L(^{-1}))</td>
<td>5.2 ± 0.2</td>
<td>5.2 ± 0.1</td>
</tr>
<tr>
<td>Insulin (µU·mL(^{-1}))</td>
<td>16.6 ± 2.9</td>
<td>14.1 ± 1.9</td>
</tr>
<tr>
<td>C-peptide (ng·mL(^{-1}))</td>
<td>2.7 ± 0.4</td>
<td>2.4 ± 0.4</td>
</tr>
<tr>
<td>Glucose AUC (mmol·L(^{-1})·(120 min(^{-1})))</td>
<td>13.6 ± 1.0</td>
<td>12.9 ± 0.8</td>
</tr>
<tr>
<td>Insulin AUC (µU·mL(^{-1})·(120 min(^{-1})))</td>
<td>213.5 ± 40.2</td>
<td>152.7 ± 25.6 (^{a,b})</td>
</tr>
<tr>
<td>C-peptide AUC (ng·mL(^{-1})·(120 min(^{-1})))</td>
<td>19.8 ± 1.8</td>
<td>15.7 ± 1.1</td>
</tr>
<tr>
<td>Matsuda ISI (µU·mL(^{-1}), mg·mL(^{-1}))</td>
<td>3.8 ± 0.9</td>
<td>4.2 ± 0.7(^a,(^b))</td>
</tr>
<tr>
<td>HOMA-IR (µU·mL(^{-1}), mmol·L(^{-1}))</td>
<td>4.1 ± 0.8</td>
<td>3.2 ± 0.5(^a,(^b))</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.9 ± 0.1</td>
<td>6.3 ± 0.1</td>
</tr>
<tr>
<td>Total Cholesterol (mmol·L(^{-1}))</td>
<td>4.78 ± 0.29</td>
<td>4.72 ± 0.33</td>
</tr>
<tr>
<td>HDL (mmol·L(^{-1}))</td>
<td>1.09 ± 0.10</td>
<td>1.06 ± 0.08</td>
</tr>
<tr>
<td>Triglycerides (mmol·L(^{-1}))</td>
<td>1.77 ± 0.29</td>
<td>1.56 ± 0.22</td>
</tr>
<tr>
<td>Hazard ratio (Total : HDL)</td>
<td>4.8 ± 0.6</td>
<td>4.8 ± 0.5</td>
</tr>
<tr>
<td>Leptin (pg·mL(^{-1}))</td>
<td>16364 ± 2690</td>
<td>10654 ± 1050 (^{a,b})</td>
</tr>
<tr>
<td>Adiponectin (ng·mL(^{-1}))</td>
<td>8269 ± 1287</td>
<td>7163 ± 800</td>
</tr>
<tr>
<td>Total Leukocytes (10(^{9})·L(^{-1}))</td>
<td>7.00 ± 0.40</td>
<td>6.48 ± 0.51</td>
</tr>
<tr>
<td><strong>Inflammatory Cytokines</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP (mg·L(^{-1}))</td>
<td>4.65 ± 0.82</td>
<td>3.48 ± 0.52</td>
</tr>
<tr>
<td>IL-6 (pg·mL(^{-1}))</td>
<td>2.58 ± 0.38</td>
<td>2.75 ± 0.51</td>
</tr>
<tr>
<td>IL-10 (pg·mL(^{-1}))</td>
<td>2.38± 0.18</td>
<td>2.29±0.23</td>
</tr>
<tr>
<td>TNF-α (pg·mL(^{-1}))</td>
<td>6.32 ± 1.44</td>
<td>7.12 ± 1.26</td>
</tr>
<tr>
<td>IL-1β (pg·mL(^{-1}))</td>
<td>0.57 ± 0.09</td>
<td>0.46 ± 0.05</td>
</tr>
<tr>
<td>IL-1ra (pg·mL(^{-1}))</td>
<td>289.3 ± 60.7</td>
<td>252.9 ± 54.5</td>
</tr>
</tbody>
</table>

Data reported for pre and post variables are based on final participant numbers used for statistical analysis: Exercise group n=11; Control group n=10.
AUC = Area under the curve; HDL = High density lipoprotein; CRP = C-reactive protein; IL = Interleukin; ra = receptor agonist
8.5: Discussion

A novel finding from the present study was that 12-weeks of sports and group-based training improved clinical risk-factors associated with the development of T2DM within previously inactive Indigenous Australian men. Primary measures showed a decrease in insulin resistance, corresponding to decreased insulin AUC and increased estimated insulin sensitivity. Moreover, positive changes also extended to the secondary outcomes in anthropometry and VO$_{2peak}$. As such, improvements in these clinical risk-factors through group and sports-based training may assist with ameliorating the future risk of developing T2DM in this group of Indigenous Australian men.

Impaired insulin secretion and action are the two main pathophysiological disturbances leading to abnormal glucose tolerance (Kuroe et al., 2003). Early phase insulin resistance (>4 HOMA-IR) was present in the exercise condition and was reduced to normal values after training. Pre-training results suggest the participants were normo-glycemic but in an insulin resistant state. As further evidence, results of the OGTT indicated that an increase in insulin secretion was required to compensate for decreased insulin sensitivity to maintain normal glucose tolerance. Insulin AUC and estimated insulin sensitivity improved with training but did not normalise. Notably, it has been shown that changes in physical activity and dietary patterns involved with reverting back to a hunter-gatherer lifestyle (i.e. 12-weeks increased physical activity and altered nutritional intake) in non-diabetic Indigenous Australians also improved, but also did not normalise the insulin response to a glucose load (O'dea, Spargo, & Akerman, 1980). We observed a similar response to exercise training in the present investigation, although there were no changes in C-peptide, insulin AUC decreased and estimated insulin sensitivity (Matsuda ISI) improved by 35%. These improvements suggest
that a sustainable long term (>12-weeks) sports-based training approach may be required to normalise insulin sensitivity within clinically obese Indigenous Australian men.

The pathogenesis of metabolic syndrome is complex, with two main potential causative factors including insulin resistance and abdominal fat distribution (central obesity) (Alberti et al., 2006; Cameron et al., 2009). Following exercise training participants showed a decrease in abdominal obesity (WC and WHR) compared to the control condition. Waist circumference is a clinically useful measure that correlates with insulin resistance and is utilised as an indicator of central obesity and risk stratification of metabolic disease (Alberti et al., 2006; Cameron et al., 2009). Notably, Indigenous Australians are reported to have preferential central fat deposition in relation to their overall weight (Kondalsamy Chennakesavan et al., 2008). Furthermore, BMI significantly underestimates overweight and obesity as assessed by WC (Kondalsamy Chennakesavan et al., 2008). Accordingly, the difference in fat deposition within the Indigenous Australians affects the risk stratification for chronic disease development based off traditional anthropometric variables. Thus, care must be taken on generalising and interpreting these anthropometric measurements across Indigenous Australian communities (Kondalsamy Chennakesavan et al., 2008). Importantly, the exercise program was successful at reducing WC, WHR and BMI, in conjunction with reduced insulin AUC and improved estimated insulin sensitivity/resistance.

Whilst causative factors of metabolic syndrome cannot be isolated to insulin resistance and central obesity, a myriad of other factors are also implicated including, a chronic systemic inflammatory state and hormonal dysregulation (Alberti et al., 2006; Brown, 2012; Maple-Brown et al., 2013). The present study showed no changes in anti- and pro-inflammatory cytokines in response to a 12-week exercise program. Pro-inflammatory cytokines TNF-α,
IL-1β and IL-6 are released from adipose tissue and stimulate the hepatic synthesis of CRP (Petersen & Pedersen, 2005; You et al., 2013), a clinical marker predictive of cardiac complications associated with atherosclerosis and metabolic abnormalities (Pearson et al., 2003). In contrast, the anti-inflammatory cytokine IL-1ra acts as an agonist to IL-1β, whilst IL-10 inhibits the production of IL-1β and TNF-α; collectively contributing to the homeostatic control of the innate immune system (Petersen & Pedersen, 2005). Notably, the lack of change within IL-1β and TNF-α may be due to the concentrations already being within the desirable ranges prior to training. Currently, there are no published exercise training interventions reporting inflammatory cytokines in Indigenous Australian populations. Thus, it is difficult to draw firm conclusions about the clinical relevance of our findings.

Regardless of ancestry, previous literature shows both positive and equivocal results regarding the effects of aerobic and resistance training on inflammatory cytokines within sedentary populations (You et al., 2013). Accordingly, reasons for the negligible inflammatory responses within the present study might extend to insufficient changes in body adiposity or dietary behaviours of the participants (Nicklas et al., 2005; You et al., 2013). Since European settlement, the traditional fibre-rich, high-protein, low saturated fat, low carbohydrate diet of many Indigenous communities has changed to high amounts of refined carbohydrates and saturated fats (Australian Institute of Health and Welfare, 2011). While we recognise the importance of changing the dietary habits within this select population (Zimmet et al., 1984), the focus of this study was to investigate the effectiveness of an exercise intervention alone. For this reason, we suggest that future research examining the inflammatory response to exercise training within Indigenous populations include dietary intervention/s specific to a fibre-rich, high-protein, low saturated fat, low carbohydrate diet within group and community settings.
Leptin and adiponectin are adipocytokines associated with the regulation of energy balance and insulin action (Bouassida et al., 2010). Specifically, adiponectin stimulates food intake and decreases energy expenditure during a fasting state, whilst leptin decreases food intake and promotes a decrease in body mass (Bouassida et al., 2010). As such, people who are obese and/or have T2DM show reduced concentrations of adiponectin and elevated concentrations of leptin (Bouassida et al., 2010). Exercise is known to effectively reduce obesity and associated adiposity, thus, the response of leptin and adiponectin in conjunction with other compounding variables (i.e. glucose metabolism, insulin sensitivity, inflammation) may explain how exercise affects obesity. Aerobic exercise training reduces fat-mass and ideally this should occur with a concomitant decrease in leptin and an increase in adiponectin concentrations; however, as shown in a recent review, this response is not consistent (Baratta et al., 2004; Bouassida et al., 2010). Whilst fat-mass is not reported in the present study, the results of the exercise condition show improved VO\textsubscript{2peak} and insulin sensitivity/resistance in conjunction with a decrease in leptin concentration and no change in adiponectin. Collectively, these findings suggest that regular exercise can positively modify leptin concentrations; however, this change in leptin with relation to change in body composition (i.e. fat-mass and muscle mass) in Indigenous Australian men requires further investigation.

8.5.1: Conclusions

In conclusion, a 12-week exercise program within Indigenous Australian men shows improvements in metabolic, anthropometric and aerobic capacity variables. The current study was developed by members of the local Indigenous community, which shows great prospect for future programs to be extended to the wider community, including youth and women. Furthermore, these findings reiterate that the development and ownership of interventions by community members and organisations are an effective way at improving clinical health.
outcomes for primary disease prevention (Rowley et al., 2000a). Indigenous populations are community focussed and therefore a group and sports-based intervention is more appropriate for collaboration and support than widespread individualised gym-based programs. Findings of the current study compliment results from a previous health and wellness program implemented within Indigenous Australian women (Canuto et al., 2012) and highlight the potential for implementing sports-based training to improve clinical risk-factors associated with T2DM in normo-glycemic, but insulin resistant Indigenous Australian men.
CHAPTER NINE

Discussion
9.1: Overview of aims

This thesis examined the effects of exercise, particularly SSG’s in inactive, middle-aged Indigenous Australian and Caucasian men. Specifically, the present thesis investigated: 1) the comparison of the glucose and inflammatory responses to an acute cycling bout between Indigenous and Caucasian Australian men; 2) the acute effects of SSG and CYC on systemic glucose and inflammatory responses within Caucasian men; 3) the inflammatory and body composition adaptations associated with 8 weeks of SSG and CYC training within Caucasian men; 4) the effects of CYC and SSG on systemic glucose regulation and content of proteins association with glucose regulation and mitochondrial biogenesis in Caucasian men; 5) the acute effects of SSG and CYC on systemic glucose and inflammatory responses within Indigenous Australian men; and 6) the inflammatory, glucose regulatory and body composition adaptations associated with 12 weeks of sports-specific training within Indigenous Australian men. A schematic overview of the ensuing discussion is presented in Figure 9.1. This overview identifies two main topics for discussion, specifically, 1) inflammation, and 2) glucose regulation. An additional focus is to investigate these main topics in coordination with adaptations of body composition and mitochondrial biogenesis. Secondly, each of these respective topics are discussed in reference to acute and chronic (mode specific) responses for each respective ancestral group. Further, and whilst admittedly often speculative, comparisons between ancestry groups for the acute exercise specific inflammatory and glucose regulatory responses are also discussed within each section.
### Chapter 9: Discussion

#### Overview of main findings

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Figure 9.1 A schematic representation of the discussion overview
9.2: Overview of main findings

Chapter 3

Study 1: Differences in post-exercise inflammatory and glucose responses between inactive Indigenous Australian and Caucasian men completing a single bout of cycle ergometry.

Aim: This study compared the acute inflammatory and glucose responses following cycle ergometry in sedentary Indigenous Australian and Caucasian men, matched for aerobic capacity and body composition.

Conclusion: Despite being matched for aerobic capacity and body composition, the Indigenous men had elevated resting TNF-α and glucose concentrations compared to the Caucasian men. The Indigenous group showed suppressed post-exercise cortisol, IL-6 and IL-ra responses when compared to the Caucasian group, despite similar aerobic capacity and exercise loads. It is suggested that the higher resting glucose and TNF-α concentration in the Indigenous group contributed to a suppressed post-exercise anti-inflammatory and glucose response to a bout of cycle ergometry.

Chapter 4

Study 2: Differences in the acute inflammatory and glucose responses between rugby-specific small-sided games and cycle ergometry in middle-aged, inactive Caucasian men.

Aim: This study compared the acute inflammatory and glucose responses within and between rugby specific SSG and CYC in sedentary, middle-aged Caucasian men.

Conclusion: Both SSG and CYC conditions were sufficient to stimulate an acute anti-inflammatory response as indicated by the post-exercise elevation of IL-6, IL-1ra and cortisol. The novel findings are that an acute bout of SSG bout is capable of inducing and
maintaining an elevated post-exercise IL-6 response and increased blood glucose disposal, compared with intensity- and duration-matched CYC condition. Accordingly, prescribing SSG as a mode of intermittent exercise may be appropriate to stimulate a sustained anti-inflammatory milieu and increased blood glucose disposal.

Chapter 5

Study 3: Rugby-specific small-sided games training is an effective alternative to improve the chronic inflammatory state compared to continuous cycle ergometry in middle-aged, inactive Caucasian men.

**Aim:** This study assessed changes in pro- and anti-inflammatory cytokines, aerobic capacity and body composition following 8-weeks of either rugby specific SSG or CYC training in middle-aged, inactive Caucasian men.

**Conclusion:** Both CYC and SSG training were similarly effective at reducing fat mass, CRP and increasing aerobic capacity. However, SSG was a more effective training stimulus to reduce IL-6 and leptin, whilst also increasing muscle mass. These results suggest that SSG may be more effective at reducing the systemic pro-inflammatory state and improving body composition when compared to CYC.
Chapter 6

**Study 4: Rugby-specific small-sided games training is an effective alternative to continuous cycle ergometry for improving glucose regulation in middle-aged, inactive Caucasian men.**

**Aim:** This study assessed systemic and skeletal muscle glucose regulatory responses following 8-weeks of either rugby specific SSG compared to CYC training in middle-aged, inactive Caucasian men.

**Conclusion:** Both exercise conditions (SSG and CYC) showed similar improvements in glucose area under the curve, fat-mass and aerobic capacity. However, SSG was more effective at increasing muscle mass, strength and decreasing insulin area under the curve. Furthermore, there were no changes in the content of skeletal muscle proteins associated with glucose regulation (i.e. GLUT4 and Akt) and mitochondrial biogenesis (i.e. PGC-1α, SIRT1, p53, COX subunits I-V and transcription factors). These results suggest that short term (8 weeks) exercise training in previously inactive men does not increase the content of these respective proteins. Rather, it is suggested that an increased efficiency of these proteins may be responsible for the systemic glucose regulatory adaptations in both CYC and SSG conditions. Additionally, the prescription of SSG may be more effective to improve insulin AUC, strength and muscle mass.
Chapter 7

Study 5: Rugby-specific small-sided games is as effective as cycle ergometry at stimulating an acute inflammatory and glucose response in middle-aged, inactive Indigenous Australian men.

Aim: This study investigated the acute effects of cycling and rugby specific SSG on inflammation and glucose regulation within an Indigenous Australian population.

Conclusion: This study identified comparable acute inflammatory and glucose responses between exercise modes of cycling and rugby SSG in an Indigenous Australian population. Although there was no increase in cortisol, both conditions stimulated a post-exercise anti-inflammatory response represented through an immediate increase in IL-6, followed by a peak in IL-ra at 60 min. No condition stimulated a post-exercise pro-inflammatory (TNF-α, IL-1β and CRP) response. The comparative acute effects of both modes combined with higher perceived enjoyment of SSG may provide Indigenous populations with a more palatable and socially-inclusive exercise intervention when compared to CYC.

Chapter 8


Aim: This study assessed the impact of a 12-week sports-based exercise intervention on glucose regulation, as well as anthropometric and inflammatory markers associated with the prevalence of T2DM in Indigenous Australian men.

Conclusion: Twelve-weeks of sports-based training in Indigenous Australian men improved aerobic capacity, body mass, BMI, WC, WHR, insulin AUC, insulin sensitivity and insulin
Chapter 9: Discussion

resistance and leptin concentrations. However, no changes in other resting pro- or anti-inflammatory cytokines were evident. These results highlight the potential for implementing sports-based training methods for improving clinical risk-factors associated with T2DM in normo-glycemic, but insulin resistant Indigenous Australian men.

9.3: Inflammation

9.3.1: Acute inflammatory responses to exercise in Caucasian men

Following exercise active skeletal muscle increases both cellular and circulating levels of IL-6 (Steensberg et al., 2000). The magnitude of the acute IL-6 response tends to be dictated by the cardiorespiratory capacity of the cohort, the extent of muscle mass involved to complete the mechanical work, as well as the intensity and duration of the exercise bout (Mendham et al., 2011; Pedersen et al., 2003; Steensberg et al., 2003). This acute increase in IL-6 is transient and responsible for an ensuing increase in the anti-inflammatory cytokine IL-1ra (as an agonist to IL-1β), hepatic synthesis of CRP, suppression of TNF-α alongside the release of cortisol (Ostrowski et al., 1999; Starkie et al., 2003; Steensberg et al., 2003). Collectively, this inflammatory process, as initiated by the exercise-induced increase in IL-6, leads to the development of a systemic anti-inflammatory milieu in response to an acute exercise bout.

An acute bout of CYC and SSG stimulated a comparable immediate post-exercise increase in IL-6 followed by an increase in IL-1ra in inactive Caucasian men (study 2). Furthermore, an immediate post-exercise increase in cortisol was evident and comparable between exercise conditions throughout the 240 min post-exercise recovery period. Despite these immediate post-exercise similarities between conditions, SSG exhibited a sustained elevation of IL-6 and IL-1ra, which was elevated above pre-exercise values at 240 min post-exercise. Given
the attempt to match intensity (i.e. perceptual and internal load via RPE and cardiovascular strain, respectively) and duration between exercise conditions, the recruitment of greater muscle mass in SSG (i.e. both upper and lower body are involved) may explain the sustained elevation in plasma IL-6 and IL-1ra when compared to CYC (Mendham et al., 2011; Ostrowski, Rohde, Zacho, Asp, & Pedersen, 1998; Pedersen et al., 2003; Steensberg et al., 2003). Specifically, the production of IL-6 in contracting skeletal muscle can account for the exercise-induced increase in plasma IL-6 (Ostrowski et al., 1998; Steensberg et al., 2000). Thus, the recruitment of greater muscle mass (i.e. upper and lower-body) during SSG has the potential to produce a higher plasma concentrations of IL-6 compared to CYC, which involves only lower-body muscle recruitment. This exercise-induced increase in IL-6 also accounts for the plasma expression of IL-1ra, which showed a sustained elevation above pre-values in only the SSG condition. Collectively, while these results indicate that both CYC and SSG induced an acute post exercise anti-inflammatory milieu, it does show SSG to be the dominant condition for maintaining this systemic anti-inflammatory state. The present study is the first to report acute systemic anti-inflammatory responses to SSG’s in inactive men, although further research is required to address potential mechanisms underlying these present findings from inflammatory signalling through the NF-κB pathways within skeletal muscle.

The anti-inflammatory response to acute exercise has been shown to inhibit the release of pro-inflammatory cytokines (Helge et al., 2003). These changes in anti-inflammatory markers within the Caucasian men (study 2) occurred in the absence of altered pro-inflammatory markers TNF-α and IL-1β. These data concur with the proposed response that the contraction-induced release of IL-6 promotes an anti-inflammatory environment by stimulating the production of IL-1ra and inhibiting the pro-inflammatory release of TNF-α.
Chapter 9: Discussion

and IL-1β following non-damaging exercise (Schindler et al., 1990; Starkie et al., 2003). In a rested state, increased IL-6 in the presence of elevated TNF-α is indicative of low-grade chronic systemic inflammation, whilst an exercise-induced increase of IL-6 in the absence of an elevated TNF-α is indicative of increased energy demand from skeletal muscle contraction (Walsh et al., 2011). The lack of changes in the plasma concentration of TNF-α and IL-1β are also reported in previous studies, and may be due to the inactive characteristics of the participants and/or the prescribed exercise intensity (Harris et al., 2008; Mendham et al., 2011). It is likely that more strenuous and longer duration exercise such as triathlons and ultra-marathons are required to stimulate immunological strain and thus an elevated post-exercise IL-1β and TNF-α (Ostrowski et al., 1999; Walsh et al., 2011). Regardless, strenuous and excessive duration of exercise are inappropriate, and hence not prescribed, to inactive middle-aged cohorts, which is one possible explanation for the lack of literature describing any change in pro-inflammatory markers in response to an acute exercise bout. Notably, CRP is reported to peak 24-48 h post-exercise in response to muscle damaging exercise modes, and represents a limitation of these studies that report up to 240 min post-exercise (Mendham et al., 2011; Neubauer et al., 2008).

9.3.2: Acute inflammatory responses to exercise in Indigenous Australian men

Despite literature supporting lifestyle and exercise interventions across different ethnic cohorts, there is no information relating to acute exercise responses within Indigenous Australians (Pan et al. 1997; Unwin et al. 2002; Zimmet et al. 2003; Rowley et al. 2000b). As such, prior to providing specific exercise training recommendations (modality, intensity and duration), research is required to quantify the acute exercise-induced inflammatory and glucose regulatory responses within Indigenous populations. Accordingly, the current thesis reports that there was no immediate post-exercise increase in cortisol and further, no
differences in exercise-induced responses between conditions (CYC and SSG) within inactive Indigenous Australian men (study 5). Furthermore, comparable increases of IL-6 and IL-1ra were evident between exercise conditions, suggesting CYC and SSG are similarly effective at promoting increased plasma concentrations of anti-inflammatory cytokines within Indigenous Australian men.

In the absence of any relevant, comparable literature, and while accepting the limitations of cross-ancestry comparisons, the noted mode-specific comparisons differed between the studies specific to the Indigenous men (study 5) and the Caucasian men (study 2). In particular, the Caucasian men sustained an elevated IL-6 and IL-1ra response in SSG when compared (within group) to the CYC condition; however the Indigenous Australian men showed similar responses (within group) between SSG and CYC conditions for all inflammatory cytokines. A possible explanation for these different mode-specific outcomes between the Indigenous and Caucasian men may be due to the different activity profiles of the respective SSG conditions. In the SSG conditions there was a significant difference between ancestry groups in the total distance covered (Indigenous, 2677 ±139; Caucasian, 3173 ±92 m; p=0.011) and mean speed (Indigenous, 66.8 ±3.5 m·min⁻¹; Caucasian, 79.2 ±2.3 m·min⁻¹; p=0.010). Accordingly, although the relative mean intensity (i.e. HR and RPE) was matched between exercise conditions (within ancestry groups), the higher external load in the SSG condition for the Caucasian group (study 2) may account for the sustained elevation of IL-6 and IL-1ra when compared to the CYC condition, thus, accounting for the conflicting mode-specific results between Caucasian and Indigenous Australian men.

In addition to the systemic anti-inflammatory response, both CYC and SSG did not stimulate a post-exercise systemic pro-inflammatory response in the Indigenous group, as measured
through plasma concentrations of TNF-α, IL-1β and CRP. Currently, there are no other studies specific to Indigenous Australian men that report acute pro-inflammatory responses to exercise. Regardless, the pro-inflammatory response in Indigenous Australian men (study 5) was similar to what was reported in the Caucasian men (study 2). As discussed previously, this pro-inflammatory response may relate directly to the lower cardio-respiratory limitations within this inactive cohort restricting the intensity and duration of the prescribed exercise bout; thus, preventing immunological strain and an associated pro-inflammatory response (Ostrowski et al., 1999; Walsh et al., 2011). Although it should be noted that previous research on acute inflammatory responses to exercise represent data either specific to Caucasian or of undisclosed ancestry population groups. Accordingly, further research is required to corroborate these results reported in Indigenous Australian men in regards to the comparative pro- and anti-inflammatory responses between groups of differing ancestry.

Regardless, an interesting finding alluded to previously, is the different mode-specific outcomes from cohorts matched in aerobic capacity and adiposity, but from different ancestry backgrounds in Australia. Differences in the activity profile between the SSG conditions demonstrate the inherent difficulties and variation in replicating these methods across inactive cohorts, thus, precluding any informative comparative statements. Of note, previous epidemiological reports have shown higher rates of chronic diseases in Indigenous cohorts when compared to their non-Indigenous counterparts (Anand et al., 2001; Miller & Cappuccio, 2007; Trewin & Madden, 2005). These reports also suggest that as part of the physiological mechanisms for the discrepancies in disease prevalence may relate to a higher systemic concentration of pro-inflammatory markers (i.e. CRP, TNF-α and IL-1β) and associated abnormalities in glucose regulation (Miller & Cappuccio, 2007). Accordingly, differences in external load in the SSG conditions may not be the only influential factor
contributing to these differing mode-specific responses within the respective ancestral groups (study 2 and 5). Therefore, to compare the pro- and anti-inflammatory response between populations of differing ancestry a standardised bout of work was required, as per the CYC loads prescribed in the respective studies.

Previous studies have suggested differences between Indigenous and non-Indigenous populations in basal or resting inflammatory and glucose regulatory characteristics (Anand et al., 2001; Miller & Cappuccio, 2007; Trewin & Madden, 2005). Furthermore, when comparing baseline characteristics between inactive Indigenous Australian and Caucasian men matched for fat-mass and aerobic capacity, results show that the Indigenous Australian men were insulin resistant and had higher resting glucose and TNF-α concentrations when compared to the Caucasian men (study 1). Additionally, in response to the acute CYC session (80-85% HR_{max}) there were no differences in the external load (i.e. pedalling resistance) between ancestry groups (Indigenous, 1.9 ±0.1 kp; Caucasian, 2.0 ±0.3 kp; p=0.80). In response to an acute cycling bout, the Indigenous men showed a suppressed anti-inflammatory response. Specifically, both groups increased anti-inflammatory cytokines IL-6 and IL-1ra immediately post-exercise. However, IL-6 remained elevated up to 60 min post-exercise and IL-1ra remained elevated up to 240 min post-exercise in the Caucasian group, but not the Indigenous group. Furthermore, the Caucasian group showed an immediate post-exercise increase in cortisol, although this was not evident in the Indigenous group. To potentially explain this observation, it is noted that glucose ingestion and thus high resting plasma glucose concentration has been shown to attenuate the IL-6 release from contracting skeletal muscle (Febbraio et al., 2003). Accordingly, the elevated resting glucose concentrations in the Indigenous Australian group may have contributed to the suppressed acute anti-inflammatory response to CYC (Fries, Hesse, Hellhammer, & Hellhammer, 2005).
However, given the small sample size and lack of previous literature, it is speculative to suggest there are ancestry dependent mechanisms responsible for this acute exercise response. Rather, this suggests that the difference in resting metabolic and inflammatory parameters of the participants representing their respective ancestry groups (although matched for aerobic capacity) contributed to these different exercise-induced responses. It remains to be shown that ancestry-dependent mechanisms are responsible for these differences in resting variables.

These are the first studies that report the acute anti- and pro-inflammatory response to rugby-specific SSG in inactive Indigenous Australian and Caucasian men. The findings show that across both cohorts of differing ancestry, an acute SSG session can be an appropriate method of exercise prescription for initiating an anti-inflammatory milieu either equal to (study 5), or better (study 2) than, a traditional bout of CYC. Furthermore, the different mode-specific outcomes between the ancestry groups may relate to the different outcomes relating to the activity profiles in the SSG conditions and/or the suppressed anti-inflammatory response reported in the Indigenous Australian men when compared to Caucasian men matched for aerobic capacity and fat-mass.

9.3.3: Inflammatory adaptations to exercise in Caucasian men

Long-term physical activity is recommended in order to reduce the chronic inflammatory state and protect against the development of chronic disease (Petersen & Pedersen, 2005). Given that the chronic inflammatory state is reportedly mediated by the ratio of adiposity to muscle mass, the extent to which the exercise mode alters these variables relating to body composition may have a bearing on altering the chronic inflammatory state (Carson et al., 2012; Fried et al., 1998; Visser et al., 2002). Such findings lend weight to the use of SSG
training in inactive populations, which have previously been shown to infer positive body composition adaptations via reduced fat-mass and increased muscle mass following 12 weeks of soccer training. Improved lean mass and reduced fat mass may in turn have a positive effect on the chronic inflammatory state (Boussida et al., 2010; Gleeson et al., 2011; Krustrup et al., 2010a; Krustrup et al., 2010b; Randers et al., 2012).

In the present thesis 8 weeks of SSG or CYC training in Caucasian men demonstrated decreased total body fat-mass and CRP concentration in both conditions (study 3). This concomitant decrease in CRP and fat-mass in response to aerobic exercise training (continuous running and/or cycling) is not uncommon in the literature (Balducci et al., 2010; Fried et al., 1998; Petersen & Pedersen, 2005). However, it must be noted that the wider literature reports inconsistent findings in regards to exercise training induced effects on CRP, with reductions occurring independently to changes in fat-mass (Donges et al., 2010; You et al., 2013). In particular, one previous study has reported that soccer specific training in hypertensive men decreased total body, gynoid and android fat-mass without any change in CRP concentration (Andersen et al., 2010b). A potential explanation for this may extend to the clinically hypertensive cohort used in that study, which was inclusive of smokers (n=5), and those on medications (statins, n=2 and anti-hypertensive medications, n=15), both of which can influence CRP concentration independent of exercise (Schaefer et al., 2005). Regardless, study 3 in the current thesis highlighted that both SSG and CYC training similarly reduced CRP concentration and decreased fat-mass; however, when considering the available literature, the association between these two variables in response to exercise training seems tenuous and requires further investigation.
Typically, chronic systemic concentrations of adipocyte derived cytokines (i.e. IL-6, TNF-α and IL-1β) stimulate an acute-phase response through the hepatic secretion of CRP (Gleeson et al., 2011; Petersen & Pedersen, 2005). In study 3, CYC and SSG training reduced CRP by 14% and 15%, respectively, though SSG was the only condition to also reduce IL-6 concentration (30%). Moreover, high resting plasma concentrations of IL-6 are associated with lower muscle mass, hence, the increase in muscle mass within the SSG condition could account for the decrease in IL-6 (Visser et al., 2002). Taken together, when compared to CYC and CON, SSG was the dominant training condition to reduce pro-inflammatory cytokines (IL-6 and CRP) alongside increased muscle mass, and may represent a reduction in multiple risk-factors associated with the development of T2DM and CVD (Pradhan et al., 2001).

Previous research reporting the response of TNF-α and IL-1β with exercise training provide equivocal findings in either healthy or diabetic participants (Balducci et al., 2010; Kadoglou et al., 2007), with no studies reporting changes following SSG training within inactive, middle-aged, men. In the current thesis, 8-weeks of SSG and CYC training within inactive middle-aged men reduced CRP and/or IL-6 concentration, although no changes were evident in TNF-α or IL-1β (study 3). In comparison, 12 months of continuous aerobic training (70-80% VO$_{2\text{max}}$) reduced CRP (28%) and IL-6 (41%), with no improvements in IL-1β, TNF-α and IL-10. These findings occurred alongside a reduction in body mass and WC, but without changes in fat- or muscle mass (Balducci et al., 2010). Conversely, 12-weeks of aerobic training at 75-80% HR$_{\text{max}}$ improved aerobic capacity and fat-mass alongside increases in TNF-α and IL-6, with no change in CRP (Donges et al., 2013). Results from these previous studies report limited data on IL-1β, and alongside results from study 3, further demonstrate the equivocal responses of inflammatory markers to aerobic training. However, it should be
Chapter 9: Discussion

noted that the modest, but significant, reduction in fat-mass in both CYC and SSG conditions may not have been adequate to mediate significant changes in TNF-α or IL-1β. Fernández-Riejos et al. (2010) demonstrate a dose-dependent leptin-induced stimulation of pro-inflammatory cytokines TNF-α and IL-6 by monocytes. Accordingly, the increase in leptin during states of inflammation strongly suggests that leptin is a part of the cytokine network that increases along with other pro-inflammatory cytokines (Fernández-Riejos et al., 2010; Meier et al., 2002). Accordingly, SSG training was the only condition to increase muscle mass, and decrease pro-inflammatory markers leptin and IL-6 in inactive Caucasian men (study 3). Many training studies have reported that leptin is only decreased in response to reduction of fat-mass (Kraemer et al., 2002; Pasman et al., 1998). However, the current results suggest that the change in leptin may have occurred independently to the relatively small changes in fat-mass. Consequently, a myriad of other mechanisms such as increased muscle mass (Visser et al., 2002), glucose regulation (Silha et al., 2003), and/or changes in other pro-inflammatory cytokines such as IL-6 (Fernández-Riejos et al., 2010) may also be implicated when interpreting the changes in resting concentrations of these pro-inflammatory cytokines. Furthermore, these confounding factors may also explain the inconsistent responses of the pro-inflammatory markers also reported above from the Caucasian population in study 3. Collectively, these results suggest no changes in TNF-α or IL-1β following either form of exercise training, although, SSG was the dominant short-term training approach for reducing leptin when compared to CYC.

An imbalance of pro- and anti-inflammatory cytokines secreted from adipose tissue contributes to metabolic dysfunction (Arita et al., 1999; Ouchi et al., 2011). Adiponectin is another hormone secreted by adipocytes that stimulates an increase in anti-inflammatory cytokines IL-10 and IL-1ra in monocytes and macrophages, while inhibiting systemic levels
of IL-6 and TNF-α (Bouassida et al., 2010; Ouchi et al., 2011). The expression of adiponectin protects against metabolic and cardiovascular disorders and is decreased in plasma and adipose tissue in obese, compared to lean individuals (Carson et al., 2012; Ouchi et al., 2011). Study 3 showed no change in anti-inflammatory markers (adiponectin, IL-1ra and IL-10) in response to SSG or CYC training in Caucasian men, despite the reduced pro-inflammatory state (i.e. CRP, IL-6 and leptin) noted earlier across SSG and CYC conditions. Furthermore, previous reports suggest a strong positive correlation between the change in IL-1ra and change in muscle mass (Meier et al., 2002). However, findings from study 3 showed no change in IL-1ra despite an increase in TB-FFM in the SSG condition. Despite the anti-inflammatory and antagonistic qualities to the pro-inflammatory marker IL-1β, there is limited evidence for the effects of aerobic training on IL-1ra (Donges et al., 2013). Moreover, previous exercise training studies report inconsistent results regarding adiponectin and IL-10, which document an increase in IL-10 in relation to fat-mass, and no change in adiponectin in response to 6 and 12 months of aerobic training, respectively (Balducci et al., 2010; Bouassida et al., 2010; Kadoglou et al., 2007). Regardless, when controlling specifically for exercise mode, study 3 reports that 8-weeks of CYC or SSG does not stimulate an increase in systemic concentrations of anti-inflammatory makers.

9.3.4: Inflammatory adaptations to exercise in Indigenous Australian men

It is well established that overweight and obese individuals have a higher resting pro-inflammatory state when compared to their leaner counterparts (Colbert et al., 2004; Fransson et al., 2010; Nguyen, Lane, Smith, & Nguyen, 2009). In particular, BMI is commonly used to estimate body adiposity, whilst abdominal obesity is inferred from measures of WC and WHR (Hu et al., 2004; Nakamura et al., 2008). However, the limitation with these measures is that they do not distinguish fat-mass from fat-free mass. Inactive Indigenous men who
undertook a sports-specific training program (study 6) did not significantly change CRP concentration, despite improved anthropometry (decreased WC, WHR and BMI). Given the absence of research literature, this study is the only exercise training intervention specific to Indigenous Australian men, and shows similar results as those recently reported in Indigenous Australian women (Canuto et al., 2012). Specifically, a 12 week structured group exercise and nutritional program in Indigenous Australian women showed no significant change in CRP (decrease of \(-0.57 \text{ mg L}^{-1}; p>0.05\)) despite a decrease in body mass and BMI, with no change in WC (Canuto et al., 2012). The current results in Indigenous Australian men (study 6) and women (Canuto et al., 2012) similarly suggest minimal change in CRP concentration. In addition, findings in another Indigenous cohort of Pacific Island adults completing 4 weeks of sports-specific training also reported no change in CRP concentration (Biddle et al., 2011; Canuto et al., 2012). A limitation of these previous studies, as with study 6, is that there are no measurements of changes in fat-mass and muscle mass alongside changes in CRP (as discussed in study 3). An analysis of such data may be required within the Indigenous Australian groups to assist in explaining the lack of change in CRP concentration in response to exercise training.

Previous exercise training studies that have reported on pro- and anti-inflammatory involve participants of undisclosed or Caucasian specific ancestry (You & Nicklas, 2008). Research specific to Indigenous Australians or other Indigenous populations have only focused on changes in systemic concentrations of CRP (Biddle et al., 2011; Canuto et al., 2012), with no previous studies specific to Indigenous Australians reporting across a range of pro- and anti-inflammatory markers. Chronic inflammatory adaptations may be a consequence of an accumulating anti-inflammatory environment in response to each respective acute exercise stimulus. However, currently there are no published exercise training interventions reporting
inflammatory cytokines in Indigenous Australian men, which therefore makes it difficult to draw firm conclusions about the clinical relevance of our findings.

In particular, study 6 only reports a decrease in leptin concentration in response to a 12-week sport-specific training program. Leptin is an important mediator of immune-related diseases and inflammatory processes (La Cava & Matarese, 2004), although it also reflects the amount of adipose tissue and muscle mass (Marshall, Grunwald, Donahoo, Scarbro, & Shetterly, 2000). Although fat-mass and muscle mass were not measured, a decrease in BMI, WC and WHR was evident, and thus demonstrates a potential mechanism for the decrease in leptin concentration within this Indigenous cohort (study 6). These findings suggest that regular exercise can positively modify leptin concentrations; however, this change in leptin with relation to change in body composition (i.e. fat-mass and muscle mass) and other pro- and anti-inflammatory markers in Indigenous Australian men requires further investigation.

Notably, it could be anticipated that the training stimulus was not adequate to stimulate inflammatory adaptations. Specifically, attendance throughout the training study was 73 ±17% (weeks 1-6) and 65 ±16% (weeks 7-12), with a mean attendance rate of 69 ±16% (0-12 weeks). This is a higher attendance than previously reported in a training program with comparable outcomes in Indigenous Australian women (Canuto et al., 2012); however, it cannot be discounted that the lack of adaptation relating to systemic inflammation resulted because the attendance to training was not sufficient. For example, Caucasian men completing 8-weeks of SSG training (study 3) with an attendance rate of 91 ±2% stimulated a reduction in CRP, IL-6 and leptin concentrations.

In summary, previous literature shows both positive and equivocal results regarding the effects of aerobic and resistance training on inflammatory cytokines within sedentary populations (Beavers et al., 2010; Nicklas et al., 2005; You et al., 2013; You & Nicklas,
Accordingly, reasons for the negligible inflammatory responses within the present study might extend to insufficient changes in body adiposity or dietary behaviours of the participants (Marshall et al., 2000). Despite other positive health outcomes (to be discussed later), a 12-week sport-specific training program was not sufficient in decreasing the pro-inflammatory state and justifies the need for a higher attendance to training sessions (similar to reported in Caucasian men - study 3) and a longer duration and/or greater training volume or intensity during the program. Whilst acknowledging the speculative nature, it could also be anticipated that the suppressed acute responses reported in study 1 may contribute to a delayed chronic inflammatory adaptation; although this hypothesis requires further investigation.

9.4: Glucose regulation

9.4.1: Acute glucose responses to exercise in Caucasian men

During exercise the increased expression of systemic anti-inflammatory markers may provide positive alterations to metabolic processes via increased fat oxidation and cellular glucose uptake (Pedersen, 2006; Pedersen & Febbraio, 2008; Walsh et al., 2011). In particular, cortisol is a stimulatory hormone that contributes to increased hepatic glucose production (Kindermann et al., 1982), and IL-6 stimulates peripheral glucose metabolism via stimulation of the AMPK pathway and associated increase in GLUT4 translocation (Carey et al., 2006; Pedersen & Febbraio, 2008). In response to an acute bout of CYC and SSG in Caucasian men (study 2), results demonstrated differences between modes regarding the glucose regulatory (and anti-inflammatory) response within the 30 – 240 min post-exercise period. Specifically, after an immediate post-exercise increase in glucose, the SSG condition showed a higher rate of glucose disposal than CYC, with plasma concentrations reduced below resting values.
Additionally, a higher rate of IL-6 stimulates the AMPK signalling pathway and associated glucose disposal through GLUT4 translocation (Helge et al., 2003). As such, the sustained elevation of IL-6 in the SSG conditions may have contributed to increased peripheral blood glucose disposal, and thus a lower plasma glucose concentration when compared to CYC (Chan et al., 2004b). Accordingly, further research is required to assess this hypothesis, particularly in relation to skeletal muscle signalling mechanisms (i.e. GLUT4-AMPK-NF-κB pathways).

Independent of the acute inflammatory response, another contributing factor to these different glucose responses may relate to an increase in HOMA-IR immediately post-exercise in the SSG condition. This increase in insulin resistance indicates an over-compensatory release of insulin to lower blood glucose concentration within the post-exercise recovery period (Goodyear & Kahn, 1998). Notably, this increased insulin resistance in the SSG condition may relate to eccentric contraction and the associated post-exercise decreased insulin sensitivity immediately post-exercise (Asp et al., 1996). Additionally, intermittent exercise of a transient but high-intensity nature is characterised by increased rates of glycolysis and associated carbohydrate metabolism and glycogen depletion (Christmass et al., 1999). Indeed, SSG incorporates eccentric exercise into frequent bouts of high-intensity sprints, separated by low intensity running over a prolonged duration. In comparison, stationary cycle ergometry is of a constant-intensity that predominately involves lower-body concentric muscular contractions. Collectively, these modes specific characteristics are what may separate the glucose y response, with further research regarding muscle damage and glycogen depletion required to explain these hypotheses.
9.4.2: Acute glucose responses to exercise in Indigenous Australian men

The acute glucose regulatory responses between SSG and CYC in Indigenous Australian men reported that SSG increased glucose, HOMA-IR and subsequent insulin concentrations immediately post-exercise (study 5). This immediate response coupled with an increase in IL-6 and a continual decrease in cortisol, collectively increased peripheral glucose metabolism and resulted in a decline in glucose below resting concentrations within the 240 min recovery period. Comparatively, CYC showed no change in glucose, insulin and HOMA-IR immediately post-exercise; however, glucose and insulin concentrations decreased below resting levels by 240 min post-exercise. As discussed previously in relation to the Caucasian men (study 2) the distinct differences in characteristics between SSG (i.e. eccentric, intermittent exercise) and CYC (i.e. concentric, continuous exercise) are likely to have resulted in differing immediate post-exercise responses, although with a similar overall state of glucose control by 240 min post-exercise. Accordingly, such distinct characteristics between modes may have the potential for justifying exercise prescription based on the needs of the client population. Regardless, an acute bout of either SSG or CYC for Indigenous or Caucasian seems an effective method to increase glucose disposal.

Recent studies examining the health status of Indigenous Australians highlights obesity, infection and/or smoking as the main causes of elevated inflammatory biomarkers and resultant chronic disease development (Rowley et al., 2003; Wang & Hoy, 2007). A comparison between systemic glucose regulatory responses between Indigenous and Caucasian men showed that when matched for aerobic capacity and adiposity the Indigneous men reported higher concentrations of fasting glucose and TNF-α (study 1). Although the findigs from the research literature are equivocal (Jiao et al., 2008), previous studies in obese, inacitve and/or clinical cohorts have reported that in a rested state a positive association exists
between elevated TNF-α and impaired glucose control (Netea et al., 1997; Nieto-Vazquez et al., 2008; Pradhan, 2007; You & Nicklas, 2008). Accordingly, higher TNF-α concentrations observed in the Indigenous group may provide a reason for the observed baseline insulin resistance (>4 HOMA-IR) and the subsequent decrease in glucose concentration immediately post-exercise (Pedersen, 2011a).

The different post-exercise glucose response to an acute CYC bout reported between Indigenous Australian and Caucasian men (study 1) may relate to the different IL-6 and cortisol responses also observed between the ancestry groups. In particular, cortisol is a stimulatory hormone that contributes to increased hepatic glucose production (Kindermann et al., 1982), and IL-6 stimulates peripheral glucose metabolism (muscle and adipose tissue) (Pedersen, 2006). As such, the combined blunted cortisol response and increased IL-6 may explain the decreasing post-exercise plasma glucose levels in the Indigenous group. In contrast, the Caucasian group showed an immediate post-exercise increase in cortisol and glucose concentrations, which may explain the sustained elevation of IL-6 following exercise (Steensberg et al., 2001a). Moreover, the higher pre-exercise glucose concentration in the Indigenous group may have accounted for the blunted cortisol response to the exercise condition (Reynolds et al., 2001). Given the small sample size and lack of previous literature, it is speculative to suggest there are ancestry dependent mechanisms responsible for these differences. However, it is suggested that the higher resting TNF-α and glucose concentrations within the Indigenous group may have contributed to the difference in post-exercise cortisol and anti-inflammatory responses between groups.

Regardless of ancestry differences to an acute exercise bout (study 1), and the different mode specific responses between Indigenous Australian (study 5) and Caucasian men (study 2); in
the context of whole body glucose disposal, a single bout of moderate-intensity exercise is important for maintaining a healthy metabolic state and should be completed daily for long-term improvements in glucose regulation, irrespective of ancestry. Accordingly, it seems either SSG via rugby is as effective mode as CYC for the purpose of glucose disposal. Of note, results from study 1 suggest it to be even more pertinent for exercise to be encouraged within the Indigenous Australian population due to their elevated resting glucose levels at a younger age, when compared to the respective Caucasian group.

9.4.3: Glucose regulatory adaptations to exercise in Caucasian men

Common measures utilised by health professionals for the diagnosis of metabolic disease include HbA1c (%A1c), fasting glucose and insulin, estimated insulin resistance (HOMA-IR), estimated insulin sensitivity (Matsuda ISI) and/or a standard 75 g OGTT (Alberti et al., 2006; Kitabchi et al., 2013; Matsuda & Defronzo, 1999). In relation to the role of exercise training to improve these markers, study 4 revealed that 8 weeks of either SSG or CYC training induced comparable improvements in HbA1c in Caucasian men - with the magnitude of decline similar to that noted from moderate-intensity endurance exercise training of ≥12weeks (Tsukui et al., 2000; Umpierre et al., 2011). However, the changes in HbA1c shown in study 4 is not a consistent finding, with multiple training studies also reporting no change in HbA1c (Cox, Burke, Morton, Beilin, & Puddley, 2004; Donges et al., 2010). Additionally, 12 weeks of SSG (soccer) training has been reported to have no significant effect on fasting glucose and insulin concentrations (Krstrup et al., 2010b; Krstrup et al., 2009; Randers et al., 2010a). An explanation for this may relate to the normo-glycemic (inferred from HOMA-IR) nature of the participants during the pre-intervention period; hence, the likelihood for exercise training to improve resting glucose and insulin
concentrations and overall insulin resistance (HOMA-IR) may be reduced (Alberti et al., 2006; Krstrup et al., 2010b; Krstrup et al., 2009; Randers et al., 2010a).

To maintain normal glucose tolerance in response to an exogenous glucose load, increased insulin secretion to compensate for decreased insulin sensitivity is required (Unwin et al., 2002). In particular, normo-glycemic Caucasian men (study 4) decreased glucose AUC and improved insulin sensitivity in response to CYC and SSG training; however, SSG was the only condition to also decrease insulin AUC. Given skeletal muscle is a major organ that is sensitive to glucose concentrations (Goodyear & Kahn, 1998), the resultant SSG-induced increase in muscle mass may have additional glucose regulatory benefits (i.e. decreased insulin AUC) compared to CYC. Collectively, for the first time we show that SSG training (study 4) in middle-aged, inactive Caucasian men is an effective training methods that can be used for improving glucose disposal and reducing insulin release in response to a standard glucose load, when compared to a more traditional exercise mode of CYC.

Improvements in insulin sensitivity and glucose uptake have shown to be facilitated by increased GLUT4 and Akt content (Gonzalez & Mcgraw, 2006; Goodyear & Kahn, 1998). An important function of Akt is to mediate the metabolic actions of insulin to stimulate cellular glucose transport (Frøsig et al., 2007; Whiteman et al., 2002). In study 4, 8 weeks of either SSG or CYC training were associated with favourable changes in blood chemistry relating to glucose disposal (i.e., OGTT AUC), despite no corresponding increases in GLUT4 or Akt protein content in skeletal muscle. A possible explanation for this result is that the improved insulin sensitivity with exercise may be more dependent on increased translocation of GLUT4 to the cell surface, rather than an increase in total GLUT4 abundance per se (Goodyear & Kahn, 1998; Hansen et al., 1998; Hawley & Lessard, 2008). In addition,
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mitochondrial biogenesis is a chronic mechanism that has shown to be partly responsible for adaptations in aerobic capacity and glucose metabolism (Baar et al., 2002). However, in the present thesis changes in skeletal muscle adaptations in the content of proteins relating to glucose regulation and mitochondrial biogenesis were not evident, despite improved whole-body glucose regulation. Other studies have reported similar findings in inactive populations following a range of exercise training durations (i.e. 2-12 weeks) and modes (i.e. resistance, HIIT cycling, continuous cycling and concurrent resistance and aerobic exercise) (Cochran et al., 2014; De Filippis et al., 2008; Donges et al., 2013; Skleryk et al., 2013). Accordingly, results from study 4, in addition to previous literature, suggest that other mechanisms independent of skeletal muscle may be responsible for the initial systemic glucose regulatory adaptation in response to both CYC and SSG training in an inactive Caucasian cohort.

9.4.4: Exercise-induced adaptations in proteins relating to mitochondrial biogenesis

The mitochondria regulates cellular glucose uptake and provides energy balance, thus, positive mitochondrial adaptations to exercise may provide a method to improve glucose tolerance (Hoppeler & Fluck, 2003). As such, the preservation of aerobic capacity (mitochondrial biogenesis) and skeletal muscle strength (lean body mass) through exercise training can ameliorate metabolic abnormalities. In part, these adaptations may result due to the extensive molecular remodelling of the skeletal muscle mitochondria (Egan & Zierath, 2013; Hoppeler & Fluck, 2003). Of further note, study 4 is the first to report adaptations of mitochondrial complexes I-V in response to SSG, although neither group (CYC or SSG) showed any significant effect of training on the protein abundance of these complexes. Both intermittent and continuous aerobic training have been associated with increases in the expression and activity of COX II and IV in healthy trained adults (Gibala et al., 2006; Little et al., 2010); however, an increase (Hood et al., 2011) or no change (Skleryk et al., 2013) has
also been reported in sedentary adults. An additional observation from study 4 is that adaptations in aerobic capacity (sub-maximal VO\textsubscript{2}) were not reciprocated by any observed changes in total protein content of mitochondrial complex (I-V) within skeletal muscle. Accordingly, the absence of changes in the protein content of the mitochondrial complexes (I-V) or matching increases to explain improved VO\textsubscript{2} during exercise suggest that the predominant training adaptations to SSG and CYC training were cardiovascular (Knoepfli-Lenzin et al., 2010), and/or increased mitochondrial activity for improved energy turnover (Egan & Zierath, 2013; Little et al., 2010).

Given the absence of significant changes in the protein content of the mitochondrial complexes, it is not surprising that there were no significant changes in other proteins associated with mitochondrial biogenesis. Both SIRT1 and p53 are two of the many proteins that have been reported to acutely regulate PGC1-\(\alpha\), and thus may contribute to training-induced mitochondrial biogenesis (Bartlett et al., 2012; Gurd et al., 2010; Little et al., 2010). In study 4 there were no significant changes in SIRT1, p53 or PGC1-\(\alpha\) protein content. Additionally, there were no changes in associated transcription factors (Tfam, NRF1, NRF2 and MEF2A) after either training intervention. There is limited literature available on the SIRT1 response to exercise in humans, with no data reporting changes in the content of proteins associated with mitochondrial biogenesis following SSG training. However, a 16% increase in PGC-1\(\alpha\) protein content has been reported following 6 weeks of interval training (Gurd et al., 2010). Interestingly, in the same study there was a 20% decrease in SIRT1 protein content, but a 31% increase in SIRT1 activity (Gurd et al., 2010). In contrast, an increase in SIRT1 (56%) content, no changes in PGC-1\(\alpha\) content, and an increase in PGC-1\(\alpha\) nuclear abundance (\(-25\%\)) has been reported in response to high-intensity interval (cycling) training (Little et al., 2010). More recently, using rat models, p53 has emerged as a potential
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acute regulator of mitochondrial content and function (Bartlett et al., 2012), although there are no published studies involving exercise training in humans. Collectively, these data suggest definitive conclusions regarding the effects of exercise on mitochondrial biogenesis in inactive populations are difficult, and thus the role of mitochondrial adaptations as mechanism for the improved glucose regulation observed in the current thesis remains equivocal.

A potential limitation of study 4 is that it does not report on GLUT4 translocation or adaptations to mitochondrial activity/efficiency. The previously discussed studies (Bartlett et al., 2012; Gibala et al., 2006; Goodyear & Kahn, 1998; Hansen et al., 1998; Hawley & Lessard, 2008; Krstrup et al., 2013; Little et al., 2010) report changes in the activity of the protein, rather than changes in total protein content in response to interval training. Consequently it is these mechanisms that may be responsible for the aerobic capacity and systemic glucose regulatory adaptations observed in study 4. Notably, following a meal, approximately one third of ingested glucose is absorbed by the liver and the remaining by peripheral tissues, primarily by skeletal muscle via insulin dependent mechanisms (Cherrington, 1999; Zierath et al., 2000). Such findings suggest that skeletal muscle is not the only glucose regulatory organ, and the improvements in systemic glucose regulation in response to a glucose load (OGTT) potentially also relate to increased functioning of other glucose sensitive organs such as the liver – though further research is required to substantiate such hypotheses.

9.4.5: Glucose regulatory adaptations to exercise in Indigenous Australian men

Training studies implemented within a range of Indigenous cohorts report ameliorating metabolic disease through reductions in glycosylated haemoglobin (HbA1c), insulin action,
body composition, blood lipids and blood pressure (Sukala et al., 2012). However, few studies report exercise interventions specific for Indigenous Australian populations (Canuto et al., 2012; Rowley et al., 2000a), with none previously reporting glucose regulation following exercise training in Indigenous Australian men. Based on the community and family-orientated culture embedded within Indigenous Australian communities (Thompson et al., 2000), group and sports-specific exercise training sessions, particularly inclusive of small-sided games (SSG) and boxing, may be an effective approach for increasing physical activity and improving clinical risk-factors associated with T2DM (Biddle et al., 2011; Canuto et al., 2012). Study 6 reports no change in HbA1c in response to 12 weeks of sports-specific training in Indigenous Australian men. Notably, recent data has shown racial and ethnic variations in HbA1c, even after adjustment for socioeconomic status, obesity, health care access and diabetes. Specifically, Black (African American), Hispanic, American Indian and Asian participants reported higher HbA1c concentrations when compared with Caucasians (Adams et al., 2008; Herman & Cohen, 2012; Saydah, Cowie, Eberhardt, De Rekeneire, & Narayan, 2007). Whilst these reports are not specific to exercise training, they do provide a hypothesis that genetic and ethnic variations to HbA1c indices may exist. Accordingly, incorporating HbA1c with other traditional measures such as fasting glucose, insulin and OGTT may be required when assessing metabolic health in groups of differing ancestry (Herman & Cohen, 2012).

Impaired insulin secretion and action are the two main pathophysiological disturbances leading to abnormal glucose tolerance (Kuroe et al., 2003). Pre-training results suggest that the Indigenous Australian participants were normo-glycemic but in an insulin resistant state (study 6). As further evidence, results of the OGTT indicated that an increase in insulin secretion was required to compensate for decreased insulin sensitivity to maintain normal
glucose tolerance. Insulin AUC and estimated insulin sensitivity improved with training but did not normalise. Notably, it has been shown that changes in physical activity and dietary patterns involved with reverting back to a hunter-gatherer lifestyle (i.e. 12-weeks increased physical activity and altered nutritional intake) in non-diabetic Indigenous Australians also improved, but also did not normalise the insulin response to a glucose load (O'dea et al., 1980). Study 6 observed a similar response to exercise training as that mentioned above, and although there were no changes in C-peptide, insulin AUC was decreased and estimated insulin sensitivity (Matsuda ISI) improved by 25%. These improvements suggest that a sustainable long term (>12-weeks) sports-based training approach may be required to normalise insulin sensitivity within clinically obese Indigenous Australian men. However, study 6 represents the first to report improved glucose regulatory adaptations in response to a community based sport-specific exercise intervention within Indigenous Australian men. Furthermore, this improved glucose regulation represents a decrease in risk-factors associated with the development of cardiovascular and metabolic abnormalities in a previously insulin resistant Indigenous Australian cohort.

9.5: Body composition and functional adaptations

9.5.1: Fat-mass and weight distribution

The accumulation of total body and abdominal body fat mass increases the chronic inflammatory state and may result in the development of metabolic abnormalities (Després & Lemieux, 2006; Gleeson et al., 2011; Ouchi et al., 2011). Inactive, middle-age Caucasian men participating in 8 weeks of SSG or CYC training resulted in a decreased total-body fat-mass of 2.6% and 2.9%, respectively (studies 3 & 4). Additionally, there was an interaction effect for a decrease in abdominal fat-mass for both training conditions as compared to CON.
condition. Moreover, despite the decrease in fat-mass there was no change in body mass, BMI, WC or WHR in response to SSG or CYC training. This variability between measures representing body composition are not uncommon in response to exercise training (Hill et al., 2007; Vazquez et al., 2007) and reiterates the obvious limitations on assumptions relating to the use of BMI, WC and WHR when assessing fat-mass and associate disease risk.

Specifically, research focusing on SSG training have assessed changes in fat-mass using DXA and report that 12 weeks of SSG (soccer) training decreased fat-mass by 3.0%, compared to continuous running (1.8%), including no change in body mass and BMI (Krustrup et al., 2010a; Krustrup et al., 2010d). This response in SSG is comparable with cycle ergometry training of similar duration and showed a 3.4% decrease in total-body fat-mass, although, conversely includes a decrease in body mass, BMI and WC (Donges et al., 2010). Collectively, a decrease in fat-mass represents a decreased in risk of developing inflammatory and glucose regulatory abnormalities with both CYC and SSG training being equally effective at stimulating this response.

For the indigenous Australian men, a limitation of the current research (study 6) was that there was no measurement for fat-mass and muscle mass through DXA analysis. Consequently, other measures of anthropometry were relied upon. In particular, WC and WHR are utilised as an indicator of central obesity and are clinically useful measures that correlate with insulin resistance and risk stratification of metabolic disease (Alberti et al., 2006; Cameron et al., 2009). Indigenous Australians have preferential central fat deposition in relation to their overall weight (Kondalsamy Chennakesavan et al., 2008) and 12 weeks of exercise training in Indigenous men (study 6) showed a decrease in BMI, WC and WHR, alongside decreased insulin AUC and leptin concentrations. Such responses concur with
previous reports of improvements in insulin AUC and leptin alongside changes in WC and BMI (Carson et al., 2012; Sillanpää et al., 2009). Comparatively, a 12 week diet and exercise program in Indigenous Australian women reported a decrease in BMI and body mass, with no change in WC, fasting glucose, HbA1c or CRP concentrations (Canuto et al., 2012). However, differences in fat deposition exist between Indigenous women and Indigenous Australian men and affect the risk stratification for chronic disease development based off traditional anthropometric variables (Kondalsamy Chennakesavan et al., 2008). Thus, care must be taken on generalising and interpreting these anthropometric measurements across Indigenous Australian communities. Overall, these improved measures of anthropometry in response to 12 weeks sport-specific training in Indigenous men represent a reduction in risk associated with the development of metabolic and cardiovascular abnormalities. However, further research may be required within Indigenous Australian men to further explain the glucose regulatory and inflammatory responses reported in study 6 in the context of changes to adiposity and body composition.

9.5.2: Muscle mass and strength

Additionally, study 5 and 6 reported that SSG training led to an increase in muscle mass and leg strength, compared to no changes observed following either the CYC or CON conditions. Previous interventions involving SSG training (12 weeks) have reported increases in maximal isometric hamstring strength and muscle mass (1.7 ±0.4 kg), when compared to prolonged treadmill running, while CYC training has shown minimal to no changes in TB-FFM (i.e. -0.6 kg and +0.7 kg) within a sedentary cohort (Balducci et al., 2010; Donges et al., 2010; Krustrup et al., 2010a; Krustrup et al., 2010b; Krustrup et al., 2010d; Samjoo et al., 2013). These previous findings, along with those of study 4, would suggest that SSG training provides sufficient eccentric loading to induce myofibrillar protein synthesis, skeletal muscle...
hypertrophy and an increase in leg strength, especially when compared to concentric
dominant contractions with the continuous CYC condition (Coffey & Hawley, 2007).
Importantly, the increased leg strength and muscle mass indicates a potential advantage of
SSG training over continuous, aerobic training to improve glucose metabolism and reduce
risk-factors associated with T2DM and CVD (Ishii et al., 1998; Jurca et al., 2005).

9.5.3: Aerobic capacity

Training intensity is an important factor for reversing risk-factors associated with the
development of metabolic abnormalities (Tjonna et al., 2008). Previous studies specific to
middle-aged, inactive men participating in SSG training report increased VO\textsubscript{2max} by 13-15%,
which was similar to other studies that report continuous training of a similar intensity (65-
85\% HR\textsubscript{max}), training period (6-16 weeks) and cohort (inactive or clinical) (Andersen et al.,
2014; Burgomaster et al., 2008; Krstrup et al., 2010a; Krstrup et al., 2010b; Schjerve et al.,
2008). In particular, an increase in VO\textsubscript{2max} between SSG (7\%) and continuous running (6\%)
occurred in the first 4 weeks of training and increased a further 6\% from 4 to 12 weeks in
only the SSG condition (Krustrup et al., 2010b). Assuming a similar progression in intensity
throughout the program, these results suggest that the cardiovascular and respiratory
adaptations are similar between conditions in the initial 4 weeks, with SSG being more
effective compared to treadmill running over 12 weeks of training. In comparison, inactive,
middle-age Caucasian men increased sub-maximal VO\textsubscript{2} between CYC and SSG conditions
(19.1\% in CYC and 18.9\% in rugby SSG; study 3 & 4). This increase in sub-maximal VO\textsubscript{2}
occurred in unison with an increase in test (GXT) duration and workload at 80\% HR\textsubscript{max},
representing the manifestation of an improved post-training aerobic capacity. These results
indicate that SSG is as effective as CYC for inducing adaptations in aerobic capacity within a
previously inactive population. Specifically, a similar aerobic capacity is obtained when
training is matched for volume and intensity, which may explain the similar results observed between condition in studies 3 and 4 (Gormley et al., 2008). Furthermore, as previously discussed these aerobic adaptations occurred independent to changes in content of mitochondrial COXI-V in skeletal muscle, and may instead indicate changes in mitochondrial activity and/or cardiovascular function within SSG and CCY conditions. However, it is clear that SSG training in previously inactive, middle-aged Caucasian men is effective in improving sub-maximal (and maximal) aerobic capacity.

Furthermore, positive adaptations of aerobic capacity (11.8%) were also observed in inactive Indigenous Australian men following exercise training (study 6). The increase in aerobic capacity within the Indigenous Australians is lower than the response noted in the Caucasian training studies (study 3 & 4). Specifically, the training program in study 6 incorporated aerobic and strength based exercises, starting at 2 sessions per week (weeks 1-6) and progressed to 3 sessions per week (weeks 7-12), while the predominately aerobic based SSG and CYC conditions in Caucasian men occurred 3 days per week for 8 weeks. These differences in training modes and training volume may reflect the differences in respective training-induced responses between the Indigenous Australian and Caucasian groups. Regardless, from a clinical perspective, men with high aerobic capacity (≥35.7 mL·kg⁻¹·min⁻¹) have shown to be nearly two-thirds less likely to develop metabolic syndrome (Laaksonen et al., 2002). Hence, the improvements in aerobic capacity noted for both ancestry groups reflect the potential for preventing the development of metabolic syndrome and associated co-morbidities within previously inactive Indigenous Australian (study 6) and Caucasian men (study 3 & 4).
Exercise as SSG requires the focus of multiple training components that include speed, endurance and strength, in a combined fashion as intermittent high-intensity sprints, interspersed by low-intensity running over a prolonged duration. Theoretically, SSG incorporates the cardiovascular, body composition, metabolic and inflammatory adaptations that can be obtained from completing both continuous and high-intensity intermittent training. For these reasons SSG as a potential model for exercise prescription in physically inactivity communities may provide a substantial physiological stimulus that is better than more traditional exercise modes solely focused on one training component.

Cycle ergometry is a part of a common exercise prescription model provided to inactive populations (Garber et al., 2011); however, it may not always represent the physical activity patterns and/or interests of specific sub-populations - in particular, the Indigenous Australian population. Accordingly, rugby specific SSG prescribed to inactive, middle-aged Indigenous Australian (study 5) and Caucasian (study 2) men was an acute stimulus that was as (study 5) or more effective (study 2) as CYC at promoting glucose disposal and a systemic anti-inflammatory milieu within the post-exercise period. Furthermore, outcomes of this thesis highlight differences in the acute inflammatory and glucose regulatory response to acute exercise in Indigenous Australian and Caucasian men completing a single bout of cycling (study 1). These results emphasize the disparity in health status and thus acute exercise response between these respective ancestry groups. Based on these acute physiological (study 1) and cultural differences, there is a need for conducting culturally appropriate training interventions designed for the respective needs of the community.
Training adaptations in inactive Caucasian men (study 3) indicate CYC and SSG training were both effective at improving CRP, VO$_2$ and fat-mass. However, differences between conditions show SSG to be a more effective training approach in reducing pro-inflammatory markers IL-6 and leptin and increasing muscle mass within inactive, middle-aged men. In the same cohort (study 4) 8 weeks of rugby-specific SSG training was also an effective alternative to CYC for improving metabolic risk-factors associated with the prevention of T2DM and CVD. In particular, study 4 revealed improvements in glycaemic control, glucose AUC, aerobic capacity, abdominal and total-body fat-mass in response to both CYC and SSG training, while SSG showed additional improvements in insulin AUC, muscle mass and lower-body strength. Despite these improvements in response to CYC and SSG training, there were no changes in the content of skeletal muscle proteins associated with glucose regulation and mitochondrial biogenesis.

Importantly, incorporating SSG across a variety of sports and group training activities into an exercise training program for Indigenous men (Study 6) improved metabolic, anthropometric and aerobic capacity variables, decreased leptin concentration, without changes in the concentrations of the remaining anti- and pro-inflammatory cytokines. This study reiterates that the development and ownership of interventions by community members and organisations are an effective way at improving clinical health outcomes for primary disease prevention. Indigenous populations are community focussed and therefore a group and sports-based intervention may be more appropriate for collaboration and support than widespread individualised gym-based programs.
CHAPTER TEN

Summary & Conclusions
Chapter 10: Summary & Conclusions

10.1: Overview

The present thesis examined the acute and chronic effects of CYC and rugby-specific SSG training on inflammation and glucose regulation within Indigenous Australian and Caucasian men. The present thesis investigated the:

1) Comparison of the glucose and inflammatory responses to an acute cycle ergometry (CYC) bout between Indigenous and Caucasian Australian men;

2) Acute effects of SSG and CYC on systemic glucose regulatory and inflammatory responses within Caucasian men;

3) Inflammatory and body composition adaptations associated with 8 weeks of SSG and CYC training within Caucasian men;

4) Effects of 8 weeks of CYC and SSG training on systemic glucose regulation and content of proteins association with glucose regulation and mitochondrial biogenesis in Caucasian men;

5) Acute effects of SSG and CYC on systemic glucose and inflammatory responses within Indigenous Australian men; and

6) Inflammatory, glucose regulatory and body composition adaptations associated with 12 weeks of sports-specific training within Indigenous Australian men.
Study 1

Differences in post-exercise inflammatory and glucose responses between inactive Indigenous Australian and Caucasian men completing a single bout of cycle ergometry.

Aim: This study compared the acute inflammatory and glucose responses following CYC in sedentary Indigenous Australian and Caucasian men, matched for aerobic capacity and body composition.

Findings

- Despite being matched for aerobic capacity and body composition, the Indigenous men had elevated resting TNF-α and glucose concentrations compared to the Caucasian men.
- The Indigenous group showed suppressed post-exercise cortisol, IL-6 and IL-ra responses when compared to the Caucasian group.

Study 2

Differences in the acute inflammatory and glucose responses between rugby-specific small-sided games and cycle ergometry in middle-aged, inactive Caucasian men.

Aim: This study compared the acute inflammatory and glucose responses within and between rugby-specific SSG and CYC in inactive, middle-aged Caucasian men.

Findings

- Both SSG and CYC conditions were sufficient to stimulate an acute anti-inflammatory response as indicated by the post-exercise elevation of IL-6, IL-1ra and cortisol.
• An acute bout of SSG bout is capable of inducing and maintaining an elevated post-exercise IL-6 response and increased blood glucose disposal, compared with intensity- and duration-matched CYC condition.

• No condition stimulated a post-exercise pro-inflammatory (TNF-α, IL-1β and CRP) response.

Study 3

*Rugby-specific small-sided games training is an effective alternative to improve the chronic inflammatory state compared to continuous cycle ergometry in middle-aged, inactive Caucasian men.*

**Aim:** This study assessed changes in pro- and anti-inflammatory cytokines, aerobic capacity and body composition following 8-weeks of either rugby-specific SSG or CYC training in middle-aged, inactive Caucasian men.

**Findings**

• Both CYC and SSG training were similarly effective at reducing fat mass, CRP and increasing aerobic capacity.

• SSG was a more effective training stimulus to reduce IL-6 and leptin, whilst also increasing muscle mass.
Study 4

*Rugby-specific small-sided games training is an effective alternative to continuous cycle ergometry for improving glucose regulation in middle-aged, inactive Caucasian men.*

**Aim:** This study assessed systemic and skeletal muscle glucose regulatory responses following 8-weeks of either rugby-specific SSG compared to CYC training in middle-aged, inactive Caucasian men.

**Findings**
- Both exercise conditions (SSG and CYC) showed similar improvements in glucose area under the curve, fat-mass and aerobic capacity.
- SSG was more effective at increasing muscle mass, strength and decreasing insulin area under the curve.
- There were no changes in the content of skeletal muscle proteins associated with glucose regulation (i.e. GLUT4 and AKT) and mitochondrial biogenesis (i.e. PGC-1α, SIRT1, p53, COX subunits I-V and transcription factors).

Study 5

*Rugby-specific small-sided games is as effective as cycle ergometry at stimulating an acute inflammatory and glucose response in middle-aged, inactive Indigenous Australian men.*

**Aim:** This study investigated the acute effects of CYC and rugby-specific SSG on inflammation and glucose responses within an Indigenous Australian population.

**Findings**
- This study identified comparable acute inflammatory and glucose responses between CYC and SSG in an Indigenous Australian population.
• Although there was no increase in cortisol, both conditions stimulated a post-exercise anti-inflammatory response, represented through an immediate increase in IL-6, followed by a peak in IL-ra at 60 min.

• No condition stimulated a post-exercise pro-inflammatory (TNF-α, IL-1β and CRP) response.

Study 6

A 12-week sports-based exercise program for inactive Indigenous Australian men improved clinical risk factors associated with type 2 diabetes.

Aim: This study assessed the impact of a 12-week sports-based exercise intervention on glucose regulation, as well as anthropometric and inflammatory markers associated with the prevalence of T2DM in Indigenous Australian men.

Findings

• Twelve-weeks of sports-based training in Indigenous Australian men improved aerobic capacity, body mass, BMI, WC, WHR, insulin AUC, insulin sensitivity and insulin resistance and leptin concentrations.

• No changes in other resting pro- or anti-inflammatory cytokines were evident.

• These results highlight the potential for implementing sports-based training methods for improving clinical risk-factors associated with T2DM in normo-glycemic, but insulin resistant Indigenous Australian men.
Chapter 10: Summary & Conclusions

10.2: Practical application

The findings from this thesis lead to several practical applications for Exercise and Health Scientists in the prescription of exercise for sedentary, middle-aged Indigenous Australian and Caucasian men, including:

- Both SSG and CYC stimulate post-exercise decreases in blood glucose concentrations in Indigenous and Caucasian groups. This highlights the value of acute moderate-intensity exercise to be completed daily, irrespective of ancestry. Although it is suggested to be even more pertinent for exercise to be encouraged within the Indigenous Australian population due to their elevated resting glucose levels at a younger age, when compared to the respective Caucasian group.

- In Caucasian men, an acute bout of SSG is capable of inducing and maintaining an elevated post-exercise IL-6 response and increased blood glucose disposal, compared with intensity- and duration-matched CYC condition.

- SSG and CYC training are both suitable exercise prescription methods in Caucasian men for reducing fat-mass, increasing aerobic capacity, decreasing glucose AUC, HbA1c, and CRP concentrations.

- When compared to CYC, SSG training is a more effective training approach in reducing insulin AUC, IL-6 and leptin concentrations, and increasing muscle mass in Caucasian men.

- 8-weeks of CYC and SSG training does not induce changes in content of skeletal muscle proteins associated with glucose regulation and mitochondrial biogenesis. It is suggested that longer training (>8 weeks) programs in previously sedentary populations may be required to induce these skeletal muscle adaptations for long-term health benefits.
• An acute SSG session is more enjoyable than cycling in Indigenous Australian men and has the benefit of creating an acute post-exercise anti-inflammatory response and blood glucose disposal.

• Group and sports-based training programs can be utilised as a primary prevention strategy for the development of T2DM and CVD in Indigenous Australian men. As evidence of this training method, study 6 showed improved blood glucose metabolism in response to a glucose load (OGTT), leptin concentrations, aerobic capacity and anthropometry within a 12-week training period.

• Exercise prescription for insulin resistant Indigenous Australian men should specifically focus on risk stratification and cultural sensitivity to ensure appropriate adaptations to exercise for chronic disease prevention.

• Members and organisations of the Indigenous community should develop exercise interventions. This creates ownership over the program and educational awareness within the community for promoting physical activity.

10.3: Future research

Based on the current findings, future research is suggested in the following areas;

• While the outcomes to study one are specific to Indigenous Australians and Caucasian men, these findings highlight the need for additional research on exercise prescription involving acute and chronic responses for other Indigenous populations at high-risk of metabolic abnormalities.

• The acute effects relating to glucose regulatory, inflammatory and mitochondrial mRNA expression and protein signalling in response to an acute bout of SSG and CYC in untrained, sedentary muscle.
Chapter 10: Summary & Conclusions

- The training response to SSG and CYC training showed positive reductions in pro-inflammatory cytokines at a systemic level. Future research should investigate these systemic changes in association with skeletal muscle changes in NF-κB inflammatory signalling pathway.

- Additional research should focus on longer (>8 weeks) training programs for adaptations in content of skeletal muscle proteins relating to glucose regulation and mitochondrial biogenesis in sedentary middle-aged men.

- The increase in muscle mass associated with SSG training provides a rationale for investigating acute and chronic signalling pathways associated with muscle hypertrophy (i.e. mTOR-ribosomal protein S6 kinases signalling).

- Differences in the acute glucose regulatory and inflammatory response between trained Indigenous Australian and Caucasian men.

- The effects of a sport-specific training approach on alteration in body composition (i.e. fat-mass and muscle mass) in Indigenous Australians.

- Future group-training programs should be extended to the wider Indigenous community, including youth and women, with a focus on inflammation and glucose regulation.
CHAPTER ELEVEN

References


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APPENDIX A:

Information sheets, informed consent forms and ethics approval
INFORMATION SHEET

This study is part of PhD research by Amy Mendham

Contact Details:
If participants have any queries before, or throughout the testing and training procedures, please contact the Chief Investigator or Supervisor on:

Miss Amy Mendham    Dr Rob Duffield
PhD student        Supervisor
Chief-Investigator   Ph 0402 456 041    Ph 63384939
Ph 0402 456 041
School of Human Movement    School of Human Movement
Charles Sturt University    Charles Sturt University
Email: amendham@csu.edu.au    rduffield@csu.edu.au

Study Title:
The acute effects of aerobic exercise and modified football on molecular skeletal muscle markers of mitochondrial function, systemic inflammation and glucose control.

Purpose of the Study:
The purpose of this study is to determine the acute effects of different exercise modes on blood and muscle based markers associated with the development and protection against cardiovascular disease (CVD), type 2 diabetes mellitus (T2DM) and obesity. Specifically, this study will investigate the post-exercise signalling process within blood based and molecular markers of skeletal muscle following aerobic (cycling) and modified football interventions. Further, this study will investigate the acute exercise-induced response relating to mitochondrial functioning, inflammation and glucose control. The results of this study will assist in recognising the benefits of various exercise modes for exercise prescription purposes when preventing or alleviating chronic disease development involving the Caucasian population within regional areas.

Experimental Procedures
• Complete a pre-exercise questionnaire (ESSA) – inspected and cleared by trained exercise physiologist
• If you require medical clearance, assistance and/or advice to participate in this study a medical practitioner will be available (free of charge) at a Bathurst Medical Centre
• All participants will be informed of all testing procedures and requirements before providing written consent for their involvement in the study.
• Participants will be required to attend one testing session one week prior to their involvement in two separate acute exercise training protocols. An outline of the testing session is provided below.
• There is a large time requirement involved in this study – If the time requirements become a burden on the participant they are free to leave the study at any time.
• During all testing procedures a medical practitioner will be present for medical advice and support. As such, any health issues identified during the course of this study will be kept confidential and the participant will be immediately informed and referred to a medical practitioner (Dr N. Meulman or Dr A. Boyko) for follow up care.

Pre-intervention Testing
All participants are required to attend a testing session in which measures of body composition and aerobic fitness will be recorded. Participants are required to arrive on the morning of testing at the CSU laboratories in a rested state (previous 10-12 hours). Participants are required to avoid alcohol and caffeine completely in the 24 hours prior to testing.

Pre-intervention testing session involves the following measures and exercise tests:
✓ Height and weight
✓ Waist and hip girth
✓ Self-Reported Questionnaire - MOS SF-36 questionnaire (Filling out this questionnaire is not compulsory and any questions or the entire form may be left blank if you feel uncomfortable at any time)
✓ Blood Pressure - measured with a blood pressure cuff and stethoscope
✓ Venous collection (c-reactive protein (CRP), HbA1c, HDL-C, Total cholesterol, Triglycerides and Glucose)
✓ Dual-Energy X-ray Absorptiometry (DXA) (for measures of body composition)
✓ Aerobic Exercise Test

DXA
Ionising radiation will be used as part of the DXA scan. Each scan will be conducted at a scanning speed of 130mm/sec and the scanning resolution will be 6.5 x 13.0mm, and the dual energy x-ray beam is in the range 35-90KeV. Based on these details, each participant will receive approximately 2 MicroSieverts on one occasion for a total body composition scan. To provide a comparison with other radiation sources and procedures a normal chest or dental x-ray typically exposes patients to approximately 50 MicroSieverts.

Aerobic Exercise Test
Participants will have their aerobic exercise capacity assessed through the completion of a maximal stationary cycling protocol. Heart rate and rate of perceived exertion (RPE) will be recorded and participants will exercise until volitional exhaustion or upon attainment of age-predicted maximum heart rate (MHR). The maximal power output will allow calculation of individual relative intensities for the aerobic protocol.
Exercise Protocols
During the testing and exercise sessions the participant’s heart rate and RPE will be recorded and monitored constantly. A trained exercise physiologist will supervise all exercise testing sessions and a telephone will also be carried by the chief investigator and supervisor for use in any emergency. If an emergency (e.g. injury) occurs the participant will be provided with appropriate medical attention by the medical physician performing the muscle biopsies. All participants will receive a follow up phone call 24 h post testing procedures to check physical and mental wellbeing, allowing the participants to voice any concerns and questions in relation to this study.

Pathological Measures
All venous blood samples will be obtained from the medial antecubital vein for the measurements of glucose, tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 receptor agonist (IL-1ra). During all exercise protocols venous blood samples will be obtained pre-exercise, immediate post, 30 min, 1h, and 4h post exercise. The pre- and 4 h post exercise sample will be obtained through venepuncture and participants will have an indwelling catheter for venous samples immediate post, 30 min and 1h post exercise following the removal of the catheter.

The use of a catheter means that despite the 5 collection time points, participants will only receive 3 needle insertions. Regarding the quantity of blood collected during the respective exercise sessions, all 5 time points (Pre-exercise, immediate post, 30min, 1h, and 4h post exercise) will require 20ml (4 x 5ml tubes), resulting in the total collection of 200ml over the 4 week research period.

Muscle Biopsy Collection
Muscle biopsy samples from the vastus lateralis will be obtained pre-exercise (on one occasion), 1h and 4h following each respective exercise protocol. Dietary and physical activity standardisation will aim to ensure that the pre-exercise biopsy is representative of the pre-exercise muscular state for the remaining exercise protocol. Collectively, over the 3 weeks a total of 5 biopsies will be obtained from each participant, pre and post exercise testing sessions. Upon arrival at the laboratory, a physician who has performed over 100 biopsy extraction procedures will prepare a site on the anterior thigh surface (~15cm above the kneecap) for the collection of a muscle biopsy. A local anaesthetic will be administered to the site (please inform the Chief Investigator or physician if you are allergic to anaesthetics), and once numb, a 5mm incision will be made with a scalpel. A special biopsy needle will then be inserted into the incision site and a small piece of muscle (about the size of a split pea) will be surgically removed. After collection, the site will be cleaned, sterilized, and wound dressings applied. As will be explained later, participants may experience some discomfort during and some soreness following muscle biopsy procedure.

The doctors performing the muscle biopsy collection include; Dr Neil Meulman (M.D) or Dr Andriy Boyko (M.D).

Dietary Requirements
All meals will be standardised 12 hours prior to exercise testing procedures, and will be replicated for all testing protocols. In addition, participants will provide information involving any food allergies that the chief investigator and supervisor need to be aware of when distributing the meals. All participants will be provided with meals (by the chief investigator and supervisor) 12 hours pre-exercise testing procedures. Following the exercise protocol, participants will be required to fast for 4 hours until a blood sample is obtained. This procedure is to ensure carbohydrate repletion does not affect the variables measured.
Aerobic Exercise Protocol
The aerobic exercise protocol will involve cycling on a Monark stationary cycle ergometer (Monark 828E, Varburg, Sweden). The protocol will involve constant-intensity cycling at 50% of maximal aerobic power output for the duration of 40min. Heart rate (Vantage NV, Polar, Finland) and RPE will be monitored throughout and following the exercise session.

Modified Football Exercise Protocol
A small-sided games protocol will involve modified football (rugby league), with subjects completing 40 min of six-a-side (6v6) modified rugby (non-contact) on a modified grass rugby league field (width: 50m; length: 70m). The session will be structured as four bouts of 8min, interspersed by 2.5min rest periods. Further a Global Positioning Satellite (GPS) (SPIetite, GPSports, Aust) unit will be worn in a harness on the back (Weighs less than 800 g) during the session to quantify distance and velocity of movement patterns during the session.

All sessions and procedures will be conducted within a group environment, thus, confidentiality cannot be maintained at all times due to the presence of other participants

Study Exclusion Criteria
- Persons involved in current or recent aerobic or resistance training;
- This includes more than one moderate to vigorous organised exercise session per week;
- Any known diagnosed stable or unstable cardiovascular or heart disease;
- Persons with a previous diagnosis of diabetes or currently being tested for diabetes;
- Current smokers (including social smokers) or smokers quitting <12 months ago;
- Persons with a body mass index above 40.0 kg/m²;
- Persons with any current illnesses such as the flu, hepatitis, etc;
- Persons being treated for dental disease;
- Persons with chronic fatigue, respiratory disease, or severe asthma;
- Persons with chronic orthopaedic limitations potentially affected by exercise procedures.

Participant identification procedures
- No photographs or video footage will be taken without written permission from the participant (name, signed and dated).
- If participants provide permission-video recording and/or photography may occur during the data collection, exercise training protocols, or testing procedures.
- Photos or video footage will be used to capture the methodology, training environment, and document evidence of study procedures and protocols.
- Care will be taken to photograph or videotape the person without a shot of their head in the footage/photo.
- Photo or video footage revealing participant identity, may be discarded or their identity may be blocked out through a visual block (i.e. blurring, black patch, etc).
- Photos or video footage will not be used in any published material, and participant names will not be associated with the video recordings.
- All results and data obtained throughout this study will be kept confidential, only the Chief Investigator and Research Supervisor will oversee data collection and collation.
- If a situation arises where data is to be viewed by other colleagues within the School of Human Movement Studies, participant confidentiality and anonymity will be maintained at all times.
Participant Benefits
The results and outcomes of the proposed research study are of importance to medical and clinical practice, exercise scientists, and other allied health professionals with direct and indirect contact with the Caucasian Australian population affected by T2DM and CVD.

The beneficial and therapeutic qualities:
- Physical and mental health
- Initiate therapeutic response following a chronic exposure to a training stimulus.
- Free blood analysis of glucose and inflammatory markers pre and post exercise. This will assist in educating the participants on the benefits of exercise throughout the different exercise protocols, whilst developing an understanding of this studies aims and objectives.
- Participants will also receive information blood pressure and exercise testing measures such as aerobic fitness levels.
- The exercise testing sessions will assist in the participant’s familiarisation of the equipment and training/testing procedures involved in playing modified football and stationary cycling.
- Encourage participants to apply knowledge and confidence for future involvement in an exercise regime and/or as an exercise participant.

Participant Risks
The participants in this study will perform 2 acute exercise sessions that will be supervised by an exercise physiologist.

- This research study involves exercise of higher intensity which may be physically demanding. It is possible that participants will experience some physical discomfort during the exercise sessions, and recovery from the respective sessions.
- All participants will be monitored for soreness, and will be guided through procedures which may alleviate any persisting discomfort.
- Participants may experience particular discomfort to areas of previous injury, and should consider their involvement in the study if a past injury is likely to reoccur.
- Participants may experience some discomfort during the administration of the local anaesthetic and (despite apparent analgesia) in the collection of the muscle biopsy.
- Blood collection is likely to be an uncomfortable experience, and all efforts will be directed to ensuring the comfort of the participant during the procedure.
- Blood collection procedures will be administered by a trained phlebotomist in a safe and reclining position.
- Participants will be required to standardise their dietary patterns prior to all testing sessions and undergo a 4 hour fast following each exercise session until the 4 hour post blood sample is obtained.
- All care will be taken to ensure a comfortable training environment is provided (room lighting, temperature, cleanliness, equipment safety, etc.), and care will be taken to avoid any potential hazards.
- The DXA scan procedure involves ionising radiation, and participants should be aware of this, and inform the Chief Investigator and/or supervisor of other recent scans (chest scan, dental scan, bone density scan, MRI, CT, DXA, etc) which may have been conducted recently.
- The DXA scan exposes the participant to 2 microSieverts (mSv) of radiation (total study dose is 8mSv). In comparison with other radiation sources, a typical chest or dental scan consists of 40-50mSv and a flight from Darwin to Perth consists of 16mSv.
A separate research application form has been submitted to the CSU Radiation Safety Committee for the use of DXA in this study, and if these procedures are deemed to be safe and of minimal risk to participants, these procedures will be approved.

**Data Collection and Research Publications**

The data collected during medical procedures, exercise testing procedures, or exercise training procedures may potentially be utilised for publication in a health/exercise science Journal.

Note: Charles Sturt University’s Ethics in Human Research Committee has approved this project. If you have any complaints or reservations about the ethical conduct of this project, you may contact the Committee through the executive Officer:

The Executive Officer, Ethics in Human Research Committee,
Academic Secretariat, Charles Sturt University,
Private Mail Bag 29, Bathurst, NSW, 2795.
Tel: (02) 6338 4628, Fax: (02) 6338 4194.

Any issues you raise will be treated in confidence and investigated fully and you will be informed of the outcome.
Informed Consent Form

The acute effects of aerobic exercise and modified football on molecular skeletal muscle markers of mitochondrial function, systemic inflammation and glucose control.

Investigator Responsibilities - Participants Rights

1) As a subject you are free to withdraw your consent to participate at any time.
2) The researchers will answer any questions you may have in regard to the study at any time.

Questions concerning the study can be directed to:

Miss Amy Mendham  Dr Rob Duffield
PhD Student       Supervisor
Chief-Investigator ph 63384939
Ph 0402456041     School of Human Movement Studies
School of Human Movement Studies
Charles Sturt University  Charles Sturt University

This study is part of PhD research by Amy Mendham
I have been provided verbally (by the Chief Investigator and/or Principal Supervisor) and in writing
(through the Study Information Sheet) with sufficient information on the study, including:

- My right to participate in this project, realising that I can withdraw at any time without being subject
to any penalty or discriminatory treatment.
- My involvement and requirements in the testing procedures within this study
- Sufficient information has been provided involving the muscle biopsy procedures, potential risks and
discomfort, and I consent to this procedure.
- Any potential risks or discomforts that I may experience during both testing procedures and exercise
sessions.
- How my confidentiality will be preserved, and how data collected from me will be used during and
after my involvement in this study.
- The use of any video footage or photographs of me taken during testing procedures or exercise
training procedures.

☐ I do not want video footage or photographs of me collected, stored, published, or used in any way.
I, (print your name) ______________________________ have read the information contained within this consent form and any questions I have asked have been answered to my satisfaction.

____________________                                ______________
Signature of Participant                                         Date

____________________                                                ______________
Signature of Chief Investigator                                              Date

If any participants have any complaints regarding the manner, in which a research project is conducted, it may be given to the researcher or, alternatively to the Executive Officer, Ethics in Human Research Committee, Charles Sturt University, Bathurst, NSW (ph 6338 4628). All study participants will be provided with a copy of the Information Sheet and Consent Form for their personal records. This study has been approved by the Ethics in Human Research Committee, CSU, Bathurst, NSW.
19 January 2011

Ms Amy Mendham
Nil, Allen House
School of Human Movement Studies
BATHURST CAMPUS

Dear Ms Mendham,

Thank you for the additional information forwarded in response to a request from the Human Research Ethics Committee.

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 “The Acute Effects Of Aerobic Exercise And Modified Football On Molecular Skeletal Muscle Markers Of Mitochondrial Function, Systemic Inflammation And Glucose Control” meets the requirements of the National Statement; and ethical approval for this research is granted for a twelve month period from 19/01/2011

The protocol number issued with respect to this project is 2011/007. 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/ehrc_annrep.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;

Version 2

FIA

www.csu.edu.au
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;

you are required to complete a Progress Report form, which can be downloaded as above, by 19/01/2012 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 or chemicals 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: Dr Bob Duffield
29 November 2010

Ms Amy Mendham
School of Human Movement Studies
N1 Allen House
BATHURST CAMPUS

Dear Ms Mendham,

RE: Proposal to undertake research which involves the use of Ionising Radiation

I am writing with respects to the proposal entitled “The acute effects of aerobic exercise and modified football on molecular skeletal muscle markers of mitochondrial function, systemic inflammation and glucose control” submitted to the Radiation Safety Committee for approval.

Your proposal has been approved by the Radiation Safety Committee at the 26 November meeting and a reference number 10/09 has been issued.

You are required to complete a Completion/Progress Report, which can be downloaded from http://www.csu.edu.au/acad_sec/safety/rsc17.doc and return it on completion of your research project or by 29/11/2011 if your research has not been completed.

The Committee wishes you well in your research and if you require any further information regarding this approval, please do not hesitate to contact me.

Yours sincerely,

Julie Hicks
Executive Officer,
Radiation Safety Committee
Office of Academic Governance
Email: radiationsafety@csu.edu.au
Ph: (02) 6338 4628
Fax: (02) 6888 4194

cc Dr Rob Duffield
INFORMATION SHEET

This study is part of PhD research by Amy Mendham, who is a PhD student at CSU.

Contact Details:
If participants have any queries before, or throughout the testing and training procedures, please contact the Chief Investigator or Supervisor on:
Miss Amy Mendham
PhD student
Ph 0402 456 041
School of Human Movement
Charles Sturt University
amendham@csu.edu.au

Dr Rob Duffield
Principal Supervisor
Ph 63384939
School of Human Movement
Charles Sturt University
rduffield@csu.edu.au

Dr Aaron Coutts
Supervisor
Ph 0427 652 815
Leisure, Sport & Tourism
University of Technology
Sydney
aaron.coutts@uts.edu.au
Study Title:

The effects of 8 weeks aerobic or modified football training on skeletal muscle and biochemistry markers of mitochondrial functioning, systemic inflammation and glucose regulation.

Purpose of the Study

The purpose of this study is to determine the chronic effects of a generic gym based exercise mode (cycle) in comparison to modified touch football on markers and signalling processes associated with the development of type II diabetes (T2DM) and cardiovascular disease (CVD). Additionally, the current study will assess changes involving, body composition, skeletal muscle glucose uptake, anti- and pro-inflammatory processes, and risk markers associated with T2DM and CVD.

Study Overview and Timeline

Participants will be required to attend two testing session prior to the commencement of exercise training, and two testing sessions after the 8 week intervention period. An outline of the testing sessions and training program is provided in the below sections.

- Information Seminar → 6th October
- Pre-intervention Testing Session 1 → 13th September – 17th October
- Pre-intervention Testing Session 2 → 21st – 23rd October
- 8 Week Training Program → 24th October – 14th December
- Post-intervention Testing Session 1 → 9th – 14th December
- Post-intervention Testing Session 2 → 17th – 19th December

Testing Session 1 (45 min)

In preparation;
- Complete the pre-exercise questionnaire (ESSA) and MOS SF-36 questionnaire
- No alcohol consumption in the prior 24 hr.
- Wear comfortable modest clothing that is suitable for exercise

Blood pressure and anthropometry

Firstly, participants will have their resting systolic and diastolic blood pressure measured and recorded with a blood pressure cuff and stethoscope. Additionally, mass, height, waist and hip girth measures will be measured to calculate BMI (mass [kg]/ height [m²]), and WHR.

Sub-maximal aerobic capacity testing

Participants will then have their aerobic exercise capacity assessed through the completion of a sub-maximal stationary cycling protocol. Heart rate will be recorded each minute throughout the protocol, and subjects exercised until volitional exhaustion or upon attainment of 80% age-predicted maximal heart rate (MHR). This aerobic fitness test will familiarise participants to the aerobic cycling protocols and will identify an initial starting point for week one of training.

Sub-maximal strength testing

Participants will then have their upper- and lower-body muscular strength assessed through the completion of a sub-maximal test protocol, in which participants will attempt ascending resistances until 3 repetitions cannot be completed.

Breakfast
At the conclusion of the aerobic exercise testing procedure, participants will be provided with a complimentary breakfast which will include cereal, toast, fruit, juice, yoghurt, and tea or coffee.

**Testing Session 2 (2.5 hours)**

**In Preparation;**
- Complete a 10 hr overnight fast (water only) prior to arriving at testing;
- No alcohol consumption in the prior 24 hr;
- Wear comfortable modest clothing that is suitable for exercise
- Ensure that all clothing is free from any metal accessories (i.e. zippers, belt buckles) which will disrupt the DXA scan images.

**Muscle Biopsy Procedure**

Upon arrival at the laboratory, a physician who has performed over 100 biopsy extraction procedures will prepare a site on the anterior thigh surface (~15cm above the kneecap) for the collection of a muscle biopsy. A local anaesthetic will be administered to the site (please inform the Chief Investigator or physician if you are allergic to anaesthetics), and once numb, a 5mm incision will be made with a scalpel. A special biopsy needle will then be inserted into the incision site and a small piece of muscle (about the size of a split pea) will be surgically removed. After collection, the site will be cleaned, sterilized, wound dressings and ice applied. As will be explained later, participants may experience some discomfort during and some soreness following muscle biopsy procedure.

**Resting blood measures and Oral Glucose Tolerance Test (OGTT)**

Participants will undergo an OGTT which is a commonly performed test used in assessing diabetes status (normal, pre-diabetes, diabetes). Participants will have an in-dwelling catheter inserted into a forearm vein and a resting blood sample will be collected for the measurement of fasting glucose, fasting insulin, cholesterol and inflammatory markers (IL-1ra, IL-1β, CRP, IL-6 and TNF-α). After the resting blood sample a 300 ml glucose solution will be consumed and blood samples (for measurement of C-peptide, insulin and glucose) will be collected from the catheter at 30 minute intervals until a 2 hours sample is obtained. During this 2 hour period the participants will have a DXA scan; reading material (newspapers, magazines) and a television will also be available for entertainment. The catheter will then be removed and the site will be cleaned and wound dressings applied.

**DXA Scan Procedure**

Whilst participants are seated during the 2 hour OGTT procedure they will be required to have a whole-body DXA scan which will take approximately 10 minutes. Participants will be assisted onto a scanning bed, and a very low-dose x-ray will be used to identify the amount of fat, muscle, and bone tissue that participants have in their torso, and upper and lower limbs. Ionising radiation will be used as part of the DXA scan. Each participant will receive approximately 2 microSieverts on two occasions (pre and post exercise training) for a total body composition scan. To provide a comparison with other radiation sources and procedures a normal chest or dental x-ray typically exposes patients to approximately 50 microSieverts. As part of everyday living, everyone is exposed to naturally occurring background radiation and receives a dose of about 2000 microSieverts each year. Participants will receive a total exposure of 4 microSieverts from the proposed DXA procedures over the duration of the study. At this dose level, no harmful effects of radiation have been demonstrated as any effect is too small to measure. According to the Australia Radiation Protection and Nuclear Safety Agency the level of risk in this project is considered minimal and is equivalent to Risk Category I (<1 ; 100,000).

**Participant Exclusion (cumulative radiation-related illness):** If you have been exposed to the more than 1000 microSieverts (over and above the natural background) within the last 12 months (International Committee of Radiological Protection). This is equivalent to approximately 20 dental or chest X-rays. If you have been exposed to this high dose of radiation please inform the chief investigator or supervisors if you have any questions or concerns involving the matter.
Breakfast
At the conclusion of the OGTT procedure, participants will be provided with a complimentary breakfast which will include cereal, toast, fruit, juice, yoghurt, and tea or coffee.

Exercise Training Programs and Control Condition
- **Aerobic training group** – 12 participants; 3 days/wk
- **Touch football training group** – 12 participants; 3 days/wk
- **Control condition** - no exercise training; will receive supervised exercise training following completion of the 8 week period.
- It is expected that training days will be Monday, Wednesday and Fridays
- Heart rate monitors will be worn during all exercise sessions to quantify load and intensity.

**Aerobic (cycling) Training Program**
The aerobic exercise program will involve participants performing continuous cycling exercise on stationary ergometers for a total of 3 sessions per week. During the 8 weeks, exercise duration will increase from 30 to 45 min, and intensity will increase from 70 to 80% of maximal heart rate. In each training session pedalling resistance, rpm and heart rate will be recorded at 5 minute intervals and at the conclusion an overall RPE will be obtained. See table below for progression, duration and intensity of the respective aerobic exercise program.

**Modified Football Training Program**
The modified football program will involve participants exercising for 3 sessions per week of touch football games. Additionally, during one training session per week GPS units will be utilised to identify any improvements in velocity and total distance covered throughout the exercise program. During the 8 weeks, total session duration will increase from 30 to 45 min, and total game play (intensity) will increase from 22 to 38 minutes. In each training session heart rate will be recorded during the rest intervals and at the conclusion an overall RPE will be obtained. See table below for progression, duration and intensity of the respective modified football program.

<table>
<thead>
<tr>
<th>Aerobic Exercise Program</th>
<th>Wk 1 – 2</th>
<th>Wk 3 – 4</th>
<th>Wk 5 – 6</th>
<th>Wk 7 – 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intensity (%MHR)</td>
<td>70</td>
<td>75</td>
<td>75</td>
<td>80</td>
</tr>
<tr>
<td>Session duration*</td>
<td>30</td>
<td>35</td>
<td>40</td>
<td>45</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Modified Football Program</th>
<th>Wk 1 – 2</th>
<th>Wk 3 – 4</th>
<th>Wk 5 – 6</th>
<th>Wk 7 – 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Session Duration*</td>
<td>30</td>
<td>35</td>
<td>40</td>
<td>45</td>
</tr>
<tr>
<td>Total Game Play*</td>
<td>22</td>
<td>28</td>
<td>32</td>
<td>38</td>
</tr>
<tr>
<td>Total Rest*</td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
</tr>
<tr>
<td>No. Rest Periods</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>No. Players</td>
<td>6v6</td>
<td>6v6</td>
<td>6v6</td>
<td>6v6</td>
</tr>
<tr>
<td>Field Size</td>
<td>40m; 60m</td>
<td>40m; 60m</td>
<td>40m; 60m</td>
<td>40m; 60m</td>
</tr>
</tbody>
</table>

MHR = maximum heart rate; * = duration in minutes; No. = number; Field Size = width (meters); length (meters); 6v6 = six-a-side, total 12 Players on field
Control Condition
The control condition will involve participants continuing their sedentary life and normal dietary and nutritional patterns for the 8 week intervention period. Participants will be provided with a dietary journal and a physical activity journal in which they will be required to document any dietary changes or physical activity patterns. Following the 8 week intervention period, participants within the control group will be invited to attend the CSU gymnasium to commence a specifically tailored exercise training program.

Participant exclusion criteria
* This study is a follow-up training study which is to be based off results and findings from a recently completed acute study (single exercise session). Participants in the acute study were Caucasian males, and to compare results this training study must only involve a Caucasian population.
  • This includes more than one moderate to vigorous organised exercise session per week;
  • Any known diagnosed stable or unstable cardiovascular or heart disease;
  • Persons with a previous diagnosis of diabetes or currently being tested for diabetes;
  • Current smokers (including social smokers) or smokers quitting <12 months ago;
  • Persons with a body mass index above 35.0 kg/m²;
  • Persons with any current illnesses such as the flu, hepatitis, etc;
  • Persons being treated for dental disease;
  • Persons with chronic fatigue, respiratory disease, or severe asthma;
  • Persons with chronic orthopaedic limitations potentially affected by exercise procedures.
  • If you have been exposed to the more than 1000 microSieverts (over and above the natural background) within the last 12 months (International Committee of Radiological Protection).

Following express permission, your general practitioner may be briefly contacted to ensure you are free from known disease.

Participant identification procedures
• No photographs or video footage will be taken without written permission from the participant (name, signed and dated).
• If participants provide permission—video recording and/or photography may occur during the data collection, exercise training protocols, or testing procedures.
• Photos or video footage will be used to capture the methodology, training environment, and document evidence of study procedures and protocols.
• Care will be taken to photograph or videotape the person without a shot of their head in the footage/photo.
• Photo or video footage revealing participant identity, may be discarded or their identity may be blocked out through a visual block (i.e. blurring, black patch, etc).
• Photos or video footage will not be used in any published material, and participant names will not be associated with the video recordings.
• All results and data obtained throughout this study will be kept confidential, only the Chief Investigator and Research Supervisor will oversee data collection and collation.
• If a situation arises where data is to be viewed by other colleagues within the School of Human Movement Studies, participant confidentiality and anonymity will be maintained at all times.

Participant Benefits
Exercise participants will receive a comprehensive amount of information regarding numerous aspects of their personal health, including changes in:
  • conventional and novel cardiovascular disease and diabetes screening markers;
  • diabetes status (i.e. normal, pre-diabetic, or diabetic) and metabolic function;
  • functional musculoskeletal capacity; increased upper- and lower-body strength;
  • functional cardiovascular capacity; lower heart rates for same pedalling resistance;
  • bodily weight and girths due to the exercise training;
whole-body muscle mass, fat mass, and bone mineral content;
free exercise training

**Participant Risks**
Participants that become involved in this study may potentially experience some risk from their involvement in some of the outlined study testing and training procedures. As such, participants need to read the below list so that they are aware of these risks prior to providing Informed Consent and becoming involved in these study procedures.

- All participants will be monitored for soreness, and will be guided through procedures which may alleviate any persisting discomfort.
- Participants may experience particular discomfort to areas of previous injury, and should consider their involvement in the study if a past injury is likely to reoccur.
- Participants may experience some discomfort during the administration of the local anaesthetic and (despite apparent analgesia) in the collection of the muscle biopsy.
- The muscle biopsy procedure is likely to be an uncomfortable experience, and soreness at the biopsy site may be experienced up to 48 hours post collection.
- Blood collection is likely to be an uncomfortable experience, and all efforts will be directed to ensuring the comfort of the participant during the procedure.
- All care will be taken to ensure a comfortable training environment is provided (room lighting, temperature, cleanliness, equipment safety, etc.), and care will be taken to avoid any potential hazards.
- The DXA scan procedure involves ionising radiation, and participants should be aware of this, and inform the Chief Investigator and/or supervisor of other recent scans (chest scan, dental scan, bone density scan, MRI, CT, DXA, etc) which may have been conducted recently.
- A separate research application form has been submitted to the CSU Radiation Safety Committee for the use of DXA in this study, and if these procedures are deemed to be safe and of minimal risk to participants, these procedures will be approved.

**Participant Care and Follow-up**
After all muscle biopsy and testing procedures, the chief investigator will ensure a follow up phone call to all participants within the 24 hour period. This will ensure all participants have appropriate follow-up care. Additionally, all participants will receive a written report and verbal feedback from the Chief Investigator regarding their testing measures. The written report will include comparisons to normal healthy reference values, and target values for those values that are outside the normal range. If testing procedures reveal an abnormality, or an imminent or new disease condition, the Chief Investigator will inform the respective participant in person, and will further notify their general practitioner of these findings and provide information regarding the suspected abnormality or disease condition.

**Data Collection and Research Publications**
The data collected during medical procedures, exercise testing procedures, or exercise training procedures may potentially be utilised for publication in a health/exercise science Journal.

Note: Charles Sturt University’s Ethics in Human Research Committee has approved this project. If you have any complaints or reservations about the ethical conduct of this project, you may contact the Committee treatment through the executive Officer:

The Executive Officer, Ethics in Human Research Committee,
Academic Secretariat, Charles Sturt University,
Private Mail Bag 29, Bathurst, NSW, 2795.
Tel: (02) 6338 4628, Fax: (02) 6338 4194.

Any issues you raise will be treated in confidence and investigated fully and you will be informed of the outcome.
Informed Consent Form

The effects of 8 weeks aerobic or modified football training on skeletal muscle markers of mitochondrial functioning, systemic inflammation and glucose regulation.

Investigator Responsibilities - Participants Rights

1) As a subject you are free to withdraw your consent to participate at any time.
2) The researchers will answer any questions you may have in regard to the study at any time.

Questions concerning the study can be directed to:
Miss Amy Mendham
PhD Student
Chief-Investigator
Ph 0402456041
School of Human
Movement Studies
Charles Sturt University

Dr Rob Duffield
Principal Supervisor
Ph 63384939
School of Human
Movement Studies
Charles Sturt University

Dr Aaron Coutts
Supervisor
Ph 0427 652 815
Leisure, Sport & Tourism
University of Technology
Sydney

This study is part of PhD research by Amy Mendham, who is a PhD student at CSU.

I have been provided verbally (by the Chief Investigator and/or Supervisor/s) and in writing (through the Study Information Sheet) with sufficient information on the study, including:

- My right to participate in this project, realising that I can withdraw at any time without being subject to any penalty or discriminatory treatment.
- My actual involvement and requirements in both the testing procedures and exercise training procedures within this study.
- Any potential risks or discomforts that I may experience during both testing procedures and exercise training procedures.
- How my confidentiality will be preserved, and how data collected from me will be used during and after my involvement in this study.
- Following express permission, your general practitioner may be briefly contacted to ensure you are free from known disease.
- The use of any video footage or photographs of me taken during testing procedures or exercise training procedures.

☐ I do not want video footage or photographs of me collected, stored, published, or used in any way.

I, (print your name) ______________________________ have read the information contained within this consent form and any questions I have asked have been answered to my satisfaction.

________________________                    ______________
Appendix A

Signature of Participant                                        Date

Signature of Chief Investigator                                 Date

If any participants have any complaints regarding the manner, in which a research project is conducted, it may be given to the researcher or, alternatively to the Executive Officer, Ethics in Human Research Committee, Charles Sturt University, Bathurst, NSW (ph 6338 4628). All study participants will be provided with a copy of the Information Sheet and Consent Form for their personal records. This study has been approved by the Ethics in Human Research Committee, CSU, Bathurst, NSW.
29 September 2011

Ms Amy Mendham
N1, Allen House
School of Human Movement Studies
BATHURST CAMPUS

Dear Ms Mendham,

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 “Effects Of 8 Weeks Aerobic Or Modified Football Training On Skeletal Muscle Markers Of Mitochondrial Functioning, Systemic Inflammation And Glucose Regulation” meets the requirements of the National Statement; and ethical approval for this research is granted for a twelve month period from 29/09/2011.

The protocol number issued with respect to this project is 2011/113. 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/ehrc_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;

Version 2

FIA
• 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;
• you are required to complete a Progress Report form, which can be downloaded as above, by 29/09/2012 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

[Signature]

Julie Hicks
Executive Officer
Human Research Ethics Committee
Direct Telephone: (02) 6338 4628
Email: ethics@csu.edu.au
Cc: Dr Rob Duffield
26 July 2011

Ms Amy Mendham
Building N1, Allen House
Bathurst Campus

Dear Ms Mendham,

RE: Proposal to undertake research which involves the use of ionising Radiation

I am writing with respects to the proposal entitled "Effects of 8 weeks aerobic or modified football training on skeletal muscle markers of mitochondrial functioning, systemic inflammation and glucose regulation." submitted to the Radiation Safety Committee for approval.

Your proposal has been approved by the Radiation Safety Committee at the 22/07/11 meeting and a reference number 2011/09 has been issued.

You are required to complete a Completion/Progress Report, which can be downloaded from http://www.csu.edu.au/acad_sec/safety/rsc17.doc and return it on completion of your research project or by 1 August 2012 if your research has not been completed.

The Committee wishes you well in your research and if you require any further information regarding this approval, please do not hesitate to contact me.

Yours sincerely

[Signature]

Clare Jonker
Executive Officer,
Radiation Safety Committee
Email: radiationsafety@csu.edu.au
Ph: (02) 6338 4247
Fax: (02) 6888 4194

cc Dr Rob Duffield  Associate Professor Aaron Coutts
This study is part of PhD research by Amy Mendham

**Contact Details:**
If participants have any queries before, or throughout the testing and training procedures, please contact the Chief Investigator or Supervisor on:

Miss Amy Mendham  
PhD student  
Ph 0402 456 041  
School of Human Movement  
Charles Sturt University  
Email: amendham@csu.edu.au

Dr Rob Duffield  
Supervisor  
Ph 63384939  
School of Human Movement  
Charles Sturt University  
Email: rduffield@csu.edu.au

**Study Title:**

The acute effects of aerobic exercise and modified football on glucose control and systemic inflammation within an Indigenous Australian population

**Purpose of the Study:**
The purpose of this study is to determine the acute effects of blood based markers associated with the development and protection against cardiovascular disease (CVD), type 2 diabetes mellitus (T2DM) and obesity. Specifically, this study will investigate aerobic exercise (cycling) and modified football on glucose regulation and inflammatory processes associated with the post-exercise response within an Indigenous Australian population. The results of this study will assist in recognising the benefits of various exercise modes for exercise prescription purposes when preventing or alleviating chronic disease development involving the Indigenous population within regional and isolated areas.

**Experimental Procedures**
Consultation with local medical physician - For medical clearance you must inform your doctor on:

- Any previous injuries
- Dietary restrictions
• If you have known CVD or and T2DM
• Your involvement within this research project

• Complete a pre-exercise questionnaire (ESSA) – inspected and cleared by trained exercise physiologist
• All participants will be informed of all testing procedures and requirements (with assistance from a local Aboriginal Elder) before providing written consent for their involvement in the study.
• Participants will be required to attend one testing session one week prior to their involvement in two separate acute exercise training protocols. An outline of the testing session is provided below.
• There is a large time requirement involved in this study – If the time requirements become a burden on the participant they are free to leave the study at any time.

If you require support involving any aspect (eg, communication, translation, advice) of this research project the chief investigator, supervisor and/or an Aboriginal Elder will assist

Pre-intervention Testing
All participants are required to attend the testing session in which measures of body composition, and aerobic fitness will be recorded. Participants are required to arrive at the Walgett sporting field (10-12hours) in a rested state. Participants are required to avoid alcohol and caffeine completely in the 24 hours prior to testing.

Pre-intervention testing session involves the following measures and exercise tests:

✓ Height and weight
✓ Waist and hip girth
✓ Self-Reported Questionnaire - MOS SF-36 questionnaire
✓ Blood Pressure - measured with a blood pressure cuff and stethoscope
✓ Venous collection (c-reactive protein (CRP), HbA1c, HDL-C, Total cholesterol, Triglycerides and Glucose)
✓ Dual-Energy X-ray Absorptiometry (DXA) (for measures of body composition)
✓ Aerobic Exercise Test

DXA
Ionising radiation will be used as part of the DXA scan. Each scan will be conducted at a scanning speed of 130mm/sec and the scanning resolution will be 6.5 x 13.0mm, and the dual energy x-ray beam is in the range 35-90KeV. Based on these details, each participant will receive approximately 2 microSieverts on one occasion for a total body composition scan. To provide a comparison with other radiation sources and procedures a normal chest or dental x-ray typically exposes patients to approximately 50 microSieverts. Participants will receive a total exposure of 4 microSieverts from the proposed DXA procedures over the duration of the study.

Aerobic Exercise Test
Participants will have their aerobic exercise capacity assessed through the completion of a maximal stationary cycling protocol. Heart rate and rate of perceived exertion (RPE) will be recorded and participants will exercise until volitional exhaustion or upon attainment of age-
predicted maximum heart rate (MHR). The maximal power output will allow calculation of individual relative intensities for the aerobic protocol.

**Exercise Protocols**

**Pathological Measures**

All venous blood samples will be obtained from the medial antecubital vein for the measurements of glucose, tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 receptor agonist (IL-1ra). During all exercise protocols venous blood samples will be obtained pre-exercise, immediate post, 1h, 2h, 4h and 24h post exercise. The pre-exercise sample will be obtained through venepuncture and participants will have an in-dwelling catheter for venous samples immediate post, 1h, 2h, and 4h post exercise. After the 4 hour blood sample is obtained the catheter will be removed and the 24 hour venous sample will be obtained through venepuncture.

The use of a catheter means that despite the 6 collection time points, participants will only receive 3 needle insertions. Regarding the quantity of blood collected during the respective exercise sessions, all 6 time points (Pre-exercise, immediate post, 1h, 2h, 4h, and 24h post exercise) will require 20ml (4 x 5ml tubes), resulting in the total collection of 240ml, over the 4 week research period.

**Dietary Requirements**

All meals will be standardised 24 hours prior to exercise testing and 24 hours post exercise testing procedures, meals will then be replicated for all testing protocols. All participants will be provided with meals (by the chief investigator and supervisor) 24 hours pre and post exercise testing procedures. Following the exercise protocol, participants will be required to fast for 4 hours until a blood sample is obtained. This procedure is to ensure carbohydrate repletion does not affect the variables measured. The morning following each exercise session participants will be provided with a light breakfast which must be consumed 2 hours prior to a 24 hour post exercise blood sample being obtained.

**Testing Protocols**

During the testing and exercise sessions the participant’s heart rate and RPE will be recorded and monitored constantly. A trained exercise physiologist (if required; an Aboriginal Elder) will supervise all exercise testing sessions and a telephone will also be carried by the chief investigator and supervisor for use in any emergency. If an emergency (e.g. injury) occurs the participant will be provided with appropriate medical attention by a qualified general medical practitioner at the local medical centre. A blood sample is obtained 24 hours post exercise. This additional interaction with the participants will allow for a follow up consultation to check physical and mental wellbeing, allowing the participants to voice any concerns and questions in relation to this study.

**Aerobic Exercise Protocol**

The aerobic exercise protocol will involve cycling on a Monark stationary cycle ergometer (Monark 828E, Varburg, Sweden). The protocol will involve constant-intensity cycling at 50% of maximal aerobic power output for the duration of 40min. Heart rate (Vantage NV, Polar, Finland) and RPE will be monitored throughout and following the exercise session.
**Modified Football Exercise Protocol**

A small-sided games protocol will involve modified football (rugby league), with subjects completing 40 min of six-a-side (6v6) modified rugby (non-contact) on a modified grass rugby league field (width: 50m; length: 70m). The session will be structured as four bouts of 8min, interspersed by 2.5min rest periods. Further a Global Positioning Satellite (GPS) (SPelite, GPSports, Aust) unit will be worn during the session to quantify distance and velocity of movement patterns during the session.

**Participant identification procedures**

- No photographs or video footage will be taken without written permission from the participant and/or permission from an Aboriginal Elder, if individual participants request support (name, signed and dated).
- If participants provide permission—video recording and/or photography may occur during the data collection, exercise training protocols, or testing procedures.
- Photos or video footage will be used to capture the methodology, training environment, and document evidence of study procedures and protocols.
- Care will be taken to photograph or videotape the person without a shot of their head in the footage/photo.
- Photos or video footage revealing participant identity, may be discarded or their identity may be blocked out through a visual block (i.e. blurring, black patch, etc).
- Photos or video footage will not be used in any published material, and participant names will not be associated with the video recordings.
- All results and data obtained throughout this study will be kept confidential, only the Chief Investigator, Co-investigator and Research Supervisor will oversee data collection and collation.
- If a situation arises where data is to be viewed by other colleagues within the School of Human Movement Studies, participant confidentiality and anonymity will be maintained at all times.

**Participant Benefits**

The results and outcomes of the proposed research study are of importance to medical and clinical practice, exercise scientists, and other allied health professionals with direct and indirect contact with Indigenous Australians affected by diabetes mellitus (T2DM) and cardiovascular disease (CVD).

The beneficial and therapeutic qualities:

- Physical and mental health
- Initiate therapeutic response following a chronic exposure to a training stimulus.
- Free blood analysis of glucose and inflammatory markers pre and post exercise. This will assist in educating the participants on the benefits of exercise throughout the different exercise protocols, whilst developing an understanding of this study’s aims and objectives.
- Participants will also receive information blood pressure and exercise testing measures such as aerobic fitness levels.
- The exercise testing sessions will assist in the participant’s familiarisation of the equipment and training/testing procedures involved in playing modified football and stationary cycling.
- Encourage participants to apply knowledge and confidence for future involvement in an exercise regime and/or as an exercise participant.
Participant Risks

Prior to a participant’s involvement in this study, medical clearance must be gained following consultation with a medical physician, indicating that they are free from CVD, T2DM and injury and they are in suitable condition to participate in this exercise. The participants in this study will perform 2 acute exercise sessions that will be supervised by an exercise physiologist.

- This research study involves exercise of higher intensity which may be physically demanding. It is possible that participants will experience some physical discomfort during the exercise sessions, and recovery from the respective sessions.
- All participants will be monitored for soreness, and will be guided through procedures which may alleviate any persisting discomfort.
- Participants may experience particular discomfort to areas of previous injury, and should consider their involvement in the study if a past injury is likely to recur.
- Blood collection is likely to be an uncomfortable experience, and all efforts will be directed to ensuring the comfort of the participant during the procedure.
- Blood collection procedures will be administered by a trained phlebotomist in a safe and reclining position.
- Participants will be required to standardise their dietary patterns prior to all testing sessions and undergo a 4 hour fast following each exercise session until the 4 hour post blood sample is obtained.
- All care will be taken to ensure a comfortable training environment is provided (room lighting, temperature, cleanliness, equipment safety, etc.), and care will be taken to avoid any potential hazards.

Data Collection and Research Publications

The data collected during medical procedures, exercise testing procedures, or exercise training procedures may potentially be utilised for publication in a health/exercise science Journal.

Note: Charles Sturt University’s Ethics in Human Research Committee has approved this project. If you have any complaints or reservations about the ethical conduct of this project, you may contact the Committee through the executive Officer:

The Executive Officer, Ethics in Human Research Committee,

Academic Secretariat, Charles Sturt University,
Private Mail Bag 29, Bathurst, NSW, 2795.
Tel: (02) 6338 4628, Fax: (02) 6338 4194.

Any issues you raise will be treated in confidence and investigated fully and you will be informed of the outcome.
The acute effects of aerobic exercise and modified football on glucose control and systemic inflammation within an Indigenous Australian population

Investigator Responsibilities - Participants Rights
1) As a subject you are free to withdraw your consent to participate at any time.
2) The researchers will answer any questions you may have in regard to the study at any time.

Questions concerning the study can be directed to:
Miss Amy Mendham               Dr Rob Duffield
PhD Student                     Supervisor
Chief-Investigator             School of Human Movement Studies
Ph 0402456041                  School of Human Movement Studies
School of Human Movement Studies
Charles Sturt University        Charles Sturt University

This study is part of PhD research by Amy Mendham

☐ I request an Aboriginal Elder present during testing procedures

☐ I agree to participate in this project, realising I can withdraw at any time without being subject to any penalty or discriminatory treatment.

☐ I agree that the purpose of this research and potential risks or discomforts involved with the testing procedures have been sufficiently explained to me.

☐ I also agree that research data and any photos gathered for this study may be published or taken providing my name and confidential details are withheld.

☐ I have read the aforementioned criteria, been provided with written explanations of the procedures and understand my rights as a participant.

☐ I have been screened and approved by a trained general medical practitioner for suitability to take part in this research.

☐ I do not want video footage or photographs of me collected, stored, published, or used in any way.
I, (print your name)_________________________________ have read the information contained within this consent form and any questions I have asked have been answered to my satisfaction.

_____________________                                ______________
Signature of participant                                         Date

_____________________                                                ______________
Signature of Chief Investigator                                              Date

If any participants have any complaints regarding the manner, in which a research project is conducted, it may be given to the researcher or, alternatively to the Executive Officer, Ethics in Human Research Committee, Charles Sturt University, Bathurst, NSW (ph 6338 4628). All study participants will be provided with a copy of the Information Sheet and Consent Form for their personal records. This study has been approved by the Ethics in Human Research Committee, CSU, Bathurst, NSW.
28 September 2010

Ms Amy Mendham
N1 Allen House
School of Human Movement Studies
BATHURST CAMPUS

Dear Ms Mendham,

Thank you for the additional information forwarded in response to a request from the Human Research Ethics Committee.

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 "The Acute Effects Of Aerobic Exercise And Modified Football On Glucose Control And Systemic Inflammation Within An Indigenous Australian Population" meets the requirements of the *National Statement*; and ethical approval for this research is granted for a twelve month period from 28/09/2010

The protocol number issued with respect to this project is **2010/113.** 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/ehre_annrep.doc](http://www.csu.edu.au/research/forms/ehre_annrep.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;

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www.csu.edu.au
• 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;
• you are required to complete a Progress Report form, which can be downloaded as above, by 28/09/2011 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 or chemicals 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: Dr Rob Daffield
29 November 2010

Ms Amy Mendham
School of Human Movement Studies
NI Allen House
BATHURST CAMPUS

Dear Ms Mendham,

RE: Proposal to undertake research which involves the use of Ionising Radiation

I am writing with respects to the proposal entitled “The acute effects of aerobic exercise and modified football on molecular skeletal muscle markers of mitochondrial function, systemic inflammation and glucose control” submitted to the Radiation Safety Committee for approval.

Your proposal has been approved by the Radiation Safety Committee at the 26 November meeting and a reference number 10/09 has been issued.

You are required to complete a Completion/Progress Report, which can be downloaded from http://www.csu.edu.au/acad_sec/safety/rsc17.doc and return it on completion of your research project or by 29/11/2011 if your research has not been completed.

The Committee wishes you well in your research and if you require any further information regarding this approval, please do not hesitate to contact me.

Yours sincerely,

Julie Hicks
Executive Officer,
Radiation Safety Committee
Office of Academic Governance
Email: radiationsafety@csu.edu.au
Ph: (02) 6338 4628
Fax: (02) 6888 4194

cc Dr Rob Duffield
INFORMATION SHEET

This study is part of PhD research by Amy Mendham, who is a PhD student at CSU.

Contact Details:
If participants have any queries before, or throughout the testing and training procedures, please contact the Chief Investigator or Supervisor on:

Miss Amy Mendham
PhD student
Ph 0402 456 041
School of Human Movement
Charles Sturt University
amendham@csu.edu.au

Dr Rob Duffield
Principal Supervisor
Ph 63384939
School of Human Movement
Charles Sturt University
rduffield@csu.edu.au

Dr Aaron Coutts
Supervisor
Ph 0427 652 815
Leisure, Sport & Tourism
University of Technology
Sydney
aaron.coutts@uts.edu.au
Study Title:
The effects of an aerobic or modified football training program on systemic inflammation and glucose regulation within a regional Aboriginal Australian population.

Purpose of the Study
The purpose of this study is to determine the chronic effects of a generic gym based exercise mode (cycling) in comparison to modified touch football on markers associated with the development of type II diabetes (T2DM) and cardiovascular disease (CVD) within a regional Aboriginal population. Additionally, the current study will assess changes involving, body composition, glucose regulation, anti- and pro-inflammatory processes, and risk markers associated with T2DM and CVD.

Study Overview and Timeline
Participants will be required to attend one testing session prior to the commencement of exercise training, and one testing sessions after the 8 week intervention period.

- Pre-intervention Testing Session → 15th October – 23rd October
- 8 Week Training Program → 24th October – 14th December
- Post-intervention Testing Session → 15th – 20th December

If you require support involving any aspect (eg, communication, translation, advice, transportation) of this research project the chief investigator, supervisor and/or an Aboriginal Elder will assist

Pre and post-intervention testing (2.5 hours)
In preparation;
- Complete a 10 hr overnight fast (water only) prior to arriving at testing;
- No alcohol consumption in the prior 24 hr;
- Wear comfortable modest clothing that is suitable for exercise
- Ensure that all clothing is free from any metal accessories (i.e. zippers, belt buckles) which will disrupt the DXA scan images.
- Complete pre-exercise questionnaire (ESSA) and MOS SF-36 questionnaire

Blood pressure and anthropometry
Firstly, participants will have their resting systolic and diastolic blood pressure measured and recorded with a blood pressure cuff and stethoscope. Additionally, mass, height, waist and hip girth measures will be measured to calculate BMI (mass [kg]/ height [m²]), and waist to hip ratio (WHR).

Sub-maximal aerobic capacity testing
Participants will then have their aerobic exercise capacity assessed through the completion of a sub-maximal stationary cycling protocol. Heart rate will be recorded each minute throughout the protocol, and subjects exercised until volitional exhaustion or upon attainment of 80% age-predicted maximal heart rate (MHR). This aerobic fitness test is moderate to vigorous exercise, lasting approximately 4-8 minutes. This aerobic test will familiarise participants to the aerobic cycling protocols and will identify an initial starting point for week one of training.

Sub-maximal strength testing
Participants will then have their upper- and lower-body muscular strength (Chest press and leg press) assessed through the completion of a sub-maximal test protocol, in which participants will attempt ascending resistances until 3 repetitions cannot be completed.

Resting blood measures and Oral Glucose Tolerance Test (OGTT)
Participants will undergo an OGTT which is a commonly performed test used in assessing diabetes status (normal, pre-diabetes, diabetes). Participants will have an in-dwelling catheter inserted into a forearm vein and a resting blood sample will be collected for the measurement of fasting glucose, fasting insulin, cholesterol and inflammatory markers (IL-1ra, IL-1β, CRP, IL-6 and TNF-α). After the resting blood sample a 300 ml glucose solution will be consumed and blood samples (for measurement of C-peptide, insulin and glucose) will be collected from the catheter at 30 minute intervals until a 2 hours sample is obtained. During this 2 hour period the participants will have a DXA scan; reading material (newspapers, magazines) and a television will also be available for entertainment. The catheter will then be removed and the site will be cleaned and wound dressings applied.

**DXA Scan Procedure**

Whilst participants are seated during the 2 hour OGTT procedure they will be required to have a whole-body DXA scan which will take approximately 10 minutes. Participants will be assisted onto a scanning bed, and a very low-dose x-ray will be used to identify the amount of fat, muscle, and bone tissue that participants have in their torso, and upper and lower limbs. Ionising radiation will be used as part of the DXA scan. Each participant will receive approximately 2 microSieverts on two occasions (pre and post exercise training) for a total body composition scan. To provide a comparison with other radiation sources and procedures a normal chest or dental x-ray typically exposes patients to approximately 50 microSieverts. As part of everyday living, everyone is exposed to naturally occurring background radiation and receives a dose of about 2000 microSieverts each year. Participants will receive a total exposure of 4 microSieverts from the proposed DXA procedures over the duration of the study. At this dose level, no harmful effects of radiation have been demonstrated as any effect is too small to measure. According to the Australia Radiation Protection and Nuclear Safety Agency the level of risk in this project is considered minimal and is equivalent to Risk Category I (<1 ; 100,000).

**Participant Exclusion (cumulative radiation-related illness):** If you have been exposed to the more than 1000 microSieverts (over and above the natural background) within the last 12 months (International Committee of Radiological Protection). This is equivalent to approximately 20 dental or chest X-rays. If you have been exposed to this high dose of radiation please inform the chief investigator or supervisors if you have any questions or concerns involving the matter.

**Breakfast**

At the conclusion of the OGTT procedure, participants will be provided with a complimentary breakfast which will include cereal, toast, fruit, juice, yoghurt, and tea or coffee.

**Exercise Training Programs and Control Condition**

Participants will be randomly assigned to an:

- **Aerobic training group** – 12 participants; 3 days/wk
- **Touch football training group** – 12 participants; 3 days/wk
- **Control condition** - no exercise training; will receive supervised exercise training following completion of the 8 week period.
- It is expected that training days will be Monday, Wednesday and Fridays
- Heart rate monitors will be worn during all exercise sessions to quantify load and intensity.

**Aerobic (cycling) Training Program**

The aerobic exercise program will involve participants performing continuous a cycling exercise on stationary ergometers for a total of 3 sessions per week. During the 8 weeks, exercise duration will increase from 30 to 45 min, and intensity will be increase from 70 to 80% of maximal heart rate. In
each training session pedalling resistance, rpm and heart rate will be recorded at 5 minute intervals and at the conclusion an overall rate of perceived exertion (RPE) will be obtained. See table below for progression, duration and intensity of the respective aerobic exercise program.

**Modified Football Training Program**

The modified football program will involve participants exercising for 3 sessions per week of touch football games. Additionally, during one training session per week GPS units will be utilised to identify any improvements in velocity and total distance covered throughout the exercise program. During the 8 weeks, total session duration will increase from 30 to 45 min, and total game play (intensity) will increase from 22 to 38 minutes. In each training session heart rate will be recorded during the rest intervals and at the conclusion an overall RPE will be obtained. See table below for progression, duration and intensity of the respective modified football program.

<table>
<thead>
<tr>
<th>Aerobic Exercise Program</th>
<th>Wk 1 – 2</th>
<th>Wk 3 – 4</th>
<th>Wk 5 – 6</th>
<th>Wk 7 – 8</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intensity (%MHR)</strong></td>
<td>70</td>
<td>75</td>
<td>75</td>
<td>80</td>
</tr>
<tr>
<td><strong>Session duration</strong></td>
<td>30</td>
<td>35</td>
<td>40</td>
<td>45</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Modified Football Program</th>
<th>Wk 1 – 2</th>
<th>Wk 3 – 4</th>
<th>Wk 5 – 6</th>
<th>Wk 7 – 8</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Session Duration</strong></td>
<td>30</td>
<td>35</td>
<td>40</td>
<td>45</td>
</tr>
<tr>
<td><strong>Total Game Play</strong></td>
<td>22</td>
<td>28</td>
<td>32</td>
<td>38</td>
</tr>
<tr>
<td><strong>Total Rest</strong></td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
</tr>
<tr>
<td><strong>No. Rest Periods</strong></td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td><strong>No. Players</strong></td>
<td>6v6</td>
<td>6v6</td>
<td>6v6</td>
<td>6v6</td>
</tr>
<tr>
<td><strong>Field Size</strong></td>
<td>40m; 60m</td>
<td>40m; 60m</td>
<td>40m; 60m</td>
<td>40m; 60m</td>
</tr>
</tbody>
</table>

MHR = maximum heart rate; * = duration in minutes; No. = number; Field Size = width (meters); length (meters); 6v6 = six-a-side, total 12 Players on field

**Control Condition**

The control condition will involve participants continuing their sedentary life and normal dietary and nutritional patterns for the 8 week intervention period. Participants will be provided with a dietary journal and a physical activity journal in which they will be required to document any dietary changes or physical activity patterns. Following the 8 week intervention period, participants within the control group will be invited to attend the CSU gymnasium to commence a specifically tailored exercise training program (Free of charge).

**Participant exclusion criteria**

- This includes more than one moderate to vigorous organised exercise session per week;
- Any known diagnosed stable or unstable cardiovascular or heart disease;
- Persons with a previous diagnosis of diabetes or currently being tested for diabetes;
- Current smokers (including social smokers) or smokers quitting <12 months ago;
- Persons with a body mass index above 35.0 kg/m²;
- Persons with any current illnesses such as the flu, hepatitis, etc;
- Persons being treated for dental disease;
- Persons with chronic fatigue, respiratory disease, or severe asthma;
- Persons with chronic orthopaedic limitations potentially affected by exercise procedures.
If you have been exposed to the more than 1000 microSieverts (over and above the natural background) within the last 12 months (International Committee of Radiological Protection).

**Participant identification procedures**

- No photographs or video footage will be taken without written permission from the participant (name, signed and dated).
- If participants provide permission-video recording and/or photography may occur during the data collection, exercise training protocols, or testing procedures.
- Photos or video footage will be used to capture the methodology, training environment, and document evidence of study procedures and protocols.
- Care will be taken to photograph or videotape the person without a shot of their head in the footage/photo.
- Photo or video footage revealing participant identity, may be discarded or their identity may be blocked out through a visual block (i.e. blurring, black patch, etc).
- Photos or video footage will not be used in any published material, and participant names will not be associated with the video recordings.
- All results and data obtained throughout this study will be kept confidential, only the Chief Investigator and Research Supervisor will oversee data collection and collation.
- If a situation arises where data is to be viewed by other colleagues within the School of Human Movement Studies, participant confidentiality and anonymity will be maintained at all times.

**Participant Benefits**

Exercise participants will receive a comprehensive amount of information regarding numerous aspects of their personal health, including changes in:

- conventional and novel cardiovascular disease and diabetes screening markers;
- diabetes status (i.e. normal, pre-diabetic, or diabetic) and metabolic function;
- functional musculoskeletal capacity; increased upper- and lower-body strength;
- functional cardiovascular capacity; lower heart rates for same pedalling resistance;
- bodily weight and girths due to the exercise training;
- whole-body muscle mass, fat mass, and bone mineral content;
- free exercise training

**Participant Risks**

Participants that become involved in this study may potentially experience some risk from their involvement in some of the outlined study testing and training procedures. As such, participants need to read the below list so that they are aware of these risks prior to providing Informed Consent and becoming involved in these study procedures.

- All participants will be monitored for soreness, and will be guided through procedures which may alleviate any persisting discomfort.
- Participants may experience particular discomfort to areas of previous injury, and should consider their involvement in the study if a past injury is likely to reoccur.
- Chest pains during exercise may occur. If chest pains do occur participants must stop exercising and inform the chief investigator or supervisor immediately.
- Blood collection is likely to be an uncomfortable experience, and all efforts will be directed to ensuring the comfort of the participant during the procedure.
- All care will be taken to ensure a comfortable training environment is provided (room lighting, temperature, cleanliness, equipment safety, etc.), and care will be taken to avoid any potential hazards.
- The DXA scan procedure involves ionising radiation, and participants should be aware of this, and inform the Chief Investigator and/or supervisor of other recent scans (chest scan,
dental scan, bone density scan, MRI, CT, DXA, etc) which may have been conducted recently.

- A separate research application form has been submitted to the CSU Radiation Safety Committee for the use of DXA in this study, and if these procedures are deemed to be safe and of minimal risk to participants, these procedures will be approved.

Participant Care and Follow-up

All participants will receive a written report and verbal feedback from the Chief Investigator regarding their testing measures. The written report will include comparisons to normal healthy reference values, and target values for those values that are outside the normal range. If testing procedures reveal an abnormality, or an imminent or new disease condition, the Chief Investigator will inform the respective participant in person, and will further notify their general practitioner of these findings and provide information regarding the suspected abnormality or disease condition. If the participant does not have a preferred general practitioner then Dr Jin from Russell Street Medical Centre will be appointed to them by the chief investigator.

Data Collection and Research Publications

The data collected during medical procedures, exercise testing procedures, or exercise training procedures may potentially be utilised for publication in a health/exercise science Journal.

Note: Charles Sturt University’s Ethics in Human Research Committee has approved this project. If you have any complaints or reservations about the ethical conduct of this project, you may contact the Committee treatment through the executive Officer:

The Executive Officer, Ethics in Human Research Committee,  
Academic Secretariat, Charles Sturt University,  
Private Mail Bag 29, Bathurst, NSW, 2795.  
Tel: (02) 6338 4628, Fax: (02) 6338 4194.

Any issues you raise will be treated in confidence and investigated fully and you will be informed of the outcome.
Informed Consent Form

The effects of an aerobic or modified football training program on systemic inflammation and glucose regulation within a regional Aboriginal Australian population.

Investigator Responsibilities - Participants Rights

1) As a subject you are free to withdraw your consent to participate at any time.
2) The researchers will answer any questions you may have in regard to the study at any time.

Questions concerning the study can be directed to:
Miss Amy Mendham   Dr Rob Duffield   Dr Aaron Coutts
PhD Student       Principal Supervisor    Supervisor
Ph 0402456041      Ph 63384939       Ph 0427 652 815
School of Human    School of Human     Leisure, Sport & Tourism
Movement Studies  Movement Studies     University of Technology
Charles Sturt University  Charles Sturt University  Sydney

This study is part of PhD research by Amy Mendham who is a PhD student at CSU.
I have been provided verbally (by the Chief Investigator and/or Supervisor/s) and in writing (through the Study Information Sheet) with sufficient information on the study, including:

- My right to participate in this project, realising that I can withdraw at any time without being subject to any penalty or discriminatory treatment.
- My actual involvement and requirements in both the testing procedures and exercise training procedures within this study.
- Any potential risks or discomforts that I may experience during both testing procedures and exercise training procedures.
- How my confidentiality will be preserved, and how data collected from me will be used during and after my involvement in this study.
- The use of any video footage or photographs of me taken during testing procedures or exercise training procedures.

☐ I do not want video footage or photographs of me collected, stored, published, or used in any way.
☐ I request an Aboriginal Elder present during testing/training sessions

I, (print your name) ______________________________ have read the information contained within this consent form and any questions I have asked have been answered to my satisfaction.
If any participants have any complaints regarding the manner, in which a research project is conducted, it may be given to the researcher or, alternatively to the Executive Officer, Ethics in Human Research Committee, Charles Sturt University, Bathurst, NSW (ph 6338 4628). All study participants will be provided with a copy of the Information Sheet and Consent Form for their personal records. This study has been approved by the Ethics in Human Research Committee, CSU, Bathurst, NSW.
29 September 2011

Ms Amy Meadham
N1 Allen House
School of Human Movement Studies
BATHURST CAMPUS

Dear Ms Meadham,

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 “The Effects Of An Aerobic Or Medified Football Training Program On Systemic Inflammation And Glucose Regulation Within A Regional Aboriginal Australian Population” meets the requirements of the *National Statement*; and ethical approval for this research is granted for a twelve month period from 29/09/2011.

The protocol number issued with respect to this project is 2011/114. 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/ehre_annrep.doc](http://www.csu.edu.au/research/forms/ehre_annrep.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;

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Appendix A

- 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;
- You are required to complete a Progress Report form, which can be downloaded as above, by 29/09/2012 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: Dr Rob Duffield
26 July 2011

Ms Amy Mendham  
Building N1, Allen House  
Bathurst Campus

Dear Ms Mendham,

RE: Proposal to undertake research which involves the use of Ionising Radiation

I am writing with respects to the proposal entitled "The effects of an aerobic or modified football training program on systemic inflammation and glucose regulation within a regional Aboriginal Australian population." submitted to the Radiation Safety Committee for approval.

Your proposal has been approved by the Radiation Safety Committee at the 22/07/11 meeting and a reference number 2011/10 has been issued.

You are required to complete a Completion/Progress Report, which can be downloaded from [http://www.csu.edu.au/acad_sec/safety/rsc17.doc](http://www.csu.edu.au/acad_sec/safety/rsc17.doc) and return it on completion of your research project or by 1 August 2012 if your research has not been completed.

The Committee wishes you well in your research and if you require any further information regarding this approval, please do not hesitate to contact me.

Yours sincerely

Clare Jonker  
Executive Officer,  
Radiation Safety Committee  
Email: radiationsafety@csu.edu.au  
Ph: (02) 6338 4247  
Fax: (02) 6888 4194

cc Dr Rob Duffield  Associate Professor Aaron Coutts
APPENDIX B:

Conference Abstracts
Conference Presentations

2013 - Small-sided games training are as effective as traditional cycle ergometry at reducing clinical risk factors associated with the development of diabetes and cardiovascular disease.


Conference: European Congress of Sport Sciences, Barcelona, Spain


*Mendham AE, Duffield R, Marino Frank, Coutts, Aaron J.*

Conference: European Congress of Sport Sciences, Barcelona, Spain

2012 - The acute effects of aerobic exercise and modified rugby on inflammation and glucose homeostasis within Indigenous Australians.

*Mendham AE, Coutts AJ, Duffield R*

Conference: Exercise and Sports Science Australia, Gold Coast, QLD 2012
SMALL-SIDED GAMES TRAINING ARE AS EFFECTIVE AS TRADITIONAL CYCLE ERGOMETRY AT REDUCING CLINICAL RISK FACTORS ASSOCIATED WITH THE DEVELOPMENT OF DIABETES AND CARDIOVASCULAR DISEASE.

Mendham, AE.1, Duffield, R.2, Marino, F.1, Coutts, AJ.2, Bishop, D.3
1: CSU (Bathurst, Australia), 2: UTS (Sydney, Australia), 3: VU (Melbourne, Australia)

Introduction
The present study investigated whether small-sided games (SSG) could be an effective alternative to stationary cycling (CYC) training at reducing clinical risk factors associated with the development of type 2 diabetes mellitus (T2DM).

Method
Thirty-three middle-aged, inactive men were randomized into a CYC (n=11), SSG (n=11), or control (CON, n=11) group. Participants in exercise groups trained 3 d.wk⁻¹ for 8 weeks, while control participants maintained normal activity and dietary patterns. Pre- and post-intervention testing included a dual-energy x-ray absorptiometry scan, graded exercise test, fasting 2 h oral glucose tolerance test (OGTT) and resting muscle biopsy. Western blotting was used to assess skeletal muscle protein content for mitochondrial functioning.

Results
Both CYC and SSG increased VO₂ at 80% HR_max, and reduced glycated haemoglobin, glucose area under the curve (AUC; SSG, -14.7 ±0.1%; CYC -15.7 ±0.1%; p<0.05), and total body fat-mass compared to no change in CON (p<0.05). Only SSG reduced insulin AUC (-23.1 ±0.1%; p<0.05) and increased total body fat-free mass (p<0.05), with no change in CYC or CON (P>0.05). No significant differences were found within or between conditions for total protein content of peroxisome proliferator-activated receptor gamma coactivator -1α (PGC-1α), sirtuin-1 (SIRT1), p53, glucose transporter 4 (GLUT4), protein kinase AKT/PKB, myocyte enhancer factor 2A (MEF2A), mitochondrial transcription factor (Tfam), nuclear respiratory factor (NRF)1, NRF2 or mitochondrial complexes I-V (p>0.05).

Discussion
Intermittent exercise training via SSG is an effective alternative to continuous cycling for improving metabolic risk-factors associated with the prevention of T2DM and CVD. Despite such positive adaptations in clinical risk factors, there were no changes in the content of skeletal muscle proteins associated with glucose regulation and mitochondrial biogenesis.
EXERCISE-INDUCED INFLAMMATORY AND GLUCOSE RESPONSES IN INDIGENOUS AUSTRALIAN AND CAUCASIAN POPULATIONS.

Mendham, AE.1, Duffield, R.2, Marino, F.1, Coutts, AJ.2
1: CSU (Bathurst, Australia), 2: UTS (Sydney, Australia)

Introduction
Many Indigenous populations have increased prevalence of non-communicable chronic diseases, which contribute to a lower life expectancy compared to non-indigenous populations. However, few studies report cross-ethnicity comparative physiology that underlies these epidemiological phenomena. This study reports the acute post-exercise inflammatory and glucose responses to aerobic exercise within and between Australian Indigenous and Caucasian populations matched for fitness and body composition.

Method
Sedentary, middle-aged Indigenous (n=10) and Caucasian (n=9) Australian males volunteered to participate who were free from diagnosed cardiovascular disease or diabetes. Following baseline testing, participants completed 1 x 40 min cycle ergometry protocol at 80% maximal heart rate. Fasting venous blood was collected pre, 0, 30, 60 min and then 240 min post-exercise for analysis of glucose, insulin, cortisol, tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, IL-1 receptor agonist (ra) and C-reactive protein (CRP).

Results
Resting TNF-α and glucose concentrations were significantly (P<0.05) higher in the Indigenous group. IL-6 increased (P<0.05) from 30 to 60 min post-exercise in the Caucasian group, whilst IL-1ra concentration remained elevated 240 min post-exercise for the Caucasian (P<0.05), but not Indigenous group (P>0.05). TNF-α, IL-1β and CRP showed no exercise-induced responses within either group (P>0.05). The immediate (0 min) post-exercise cortisol and glucose increase for Caucasians was significantly (P<0.05) higher than the attenuated responses observed within the Indigenous group (P>0.05).

Discussion
The present study showed that despite the two groups representing their respective middle-aged populations and being matched for aerobic fitness and body composition, differences in baseline pro-inflammatory and glucose concentrations exist between Indigenous Australians and Caucasians. This disparity may have contributed to the post-exercise differences between groups to cycle ergometry; specifically, the blunted post-exercise anti-inflammatory (IL-6 and IL-1ra) and glucose regulatory (glucose and cortisol) response within the Indigenous group. As such, exercise interventions may be of benefit for both groups and should be tailored respective to the cultural and health characteristics present within Indigenous Australian and Caucasian populations.
THE ACUTE EFFECTS OF AEROBIC EXERCISE AND MODIFIED FOOTBALL ON INFLAMMATION AND GLUCOSE HOMEOSTASIS WITHIN INDIGENOUS AUSTRALIANS.

Mendham, Amy E 1, Coutts, Aaron J 2, and Duffield, Rob 1.

1 Charles Sturt University, School of Human Movement Studies, Bathurst, Australia
2 University of Technology Sydney, Leisure, Sport and Tourism, Sydney, Australia

Introduction: The modification of physical activity levels to reduce the risk of chronic disease development may have potential to succeed if reinforced through group participation as opposed to individualised exercise1. Team sports such as football (rugby league) are popular within Indigenous communities and may reinforce group participation and cohesion2. Thus, modified team sport activities, such as rugby small-sided games (SSG) may be an achievable alternative for low physical activity levels within rural areas1. Accordingly, the current study aimed to assess the acute effects of traditional gym based (cycling) and modified rugby (as SSG) as related to the acute regulation of inflammatory cytokines and glucose homeostasis within a regional Indigenous Australian population.

Methods: Ten non-diseased, untrained Indigenous male participants volunteered (age 38 ± 3.24 years, total body fat 27.8 ± 2.03 %). Participants were non-smokers and not clinically diagnosed with cardiovascular or metabolic disorders. Following familiarisation and a sub-maximal test to predict aerobic capacity and maximal heart rate (MHR), on two occasions participants completed two exercise protocols (cycling and SSG) of 40 min duration (4 x 10 min bouts interspersed by 2 min passive recovery) separated by 7 days recovery. The cycling protocol consisted of a target intensity of 80-85% MHR, whilst SSG consisted of non-contact rugby league of six-a-side on a modified field (width: 40 m; length: 60 m). Fasting venous blood was collected pre, post, 30 min, 60 min and 240 min post-exercise for the analysis of glucose, insulin, cortisol and inflammatory markers, tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, IL-1 receptor agonist (ra) and c-reactive protein (CRP). Heart rate (HR) was collected throughout exercise and a session rating of perceived exertion (RPE; CR100) was collected 30 min post exercise. This study was approved by the University Ethics Committee and written informed consent was obtained from all participants. All data are reported as mean ± SEM. A two-way repeated measures ANOVA was used to determine within and between condition comparisons (P<0.05).

Results: The mean HR responses for the SSG and cycling protocols were 83.1 ± 2.09% and 81.4 ± 1.51% MHR, respectively, with no significant difference between conditions (P=0.15). Additionally, session RPE was not significantly different between conditions at 50.0 ± 16.2 for SSG and 52.3 ± 4.13 for cycling (P=0.56). IL-6 and IL-1ra significantly (P<0.05) increased within the 240 min post-exercise period, with no significant differences between protocols. There were no significant changes within or between protocols for TNF-α, IL-1β and CRP (P>0.05). A comparison of insulin resistance: homeostasis model (HOMA) between resting and 240 min post-exercise shows a change from 4.50 ± 1.16 to 1.76 ± 0.53 in cycling (P=0.01) and 4.37 ± 1.20 to 1.54 ± 0.42 in SSG (P=0.02), without significance between conditions (P=0.61).

Conclusion: The present study identified similar acute inflammatory and glucose regulatory responses between cycling and SSG modes in a regional Indigenous population. Consequently, both exercise modalities may be appropriate to obtain acute responses
promoting increased health benefits involving inflammatory and glucose homeostasis. Specifically, the encouragement of SSG may provide regional Indigenous populations with a more community based physical activity intervention as opposed to individualised exercise sessions such as gym-based cycling.

References