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Short communication

Comparison of surrogate indices for insulin sensitivity with parameters of the intravenous glucose tolerance test in early lactation dairy cattle

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Abstract

The aim of this study was to investigate the correlation between different surrogate indices and parameters of the intravenous glucose tolerance test (IVGTT) in dairy cows at the start of their lactation. Ten dairy cows underwent IVGTT on days 3 to 7 after calving. Areas under the curve during the 90 min following infusion, peak and nadir concentrations, elimination rates, and times to reach half-maximal and basal concentrations for glucose, insulin, nonesterified fatty acids, and β-hydroxybutyrate were calculated. Surrogate indices were computed using the average of the IVGTT basal samples, and their correlation with the IVGTT parameters studied through the Spearman's rank test. No statistically significant or strong correlation coefficients ($P > 0.05$; $|r| < 0.50$) were observed between the insulin sensitivity measures derived from the IVGTT and any of the surrogate indices. Therefore, these results support that the assessment of insulin sensitivity in early lactation cattle cannot rely on the calculation of surrogate indices in just a blood sample, and the more laborious tests (i.e., hyperinsulinemic euglycemic clamp test or IVGTT) should be employed to predict the sensitivity of the peripheral tissues to insulin accurately.

Keywords: Dairy cow, Insulin resistance, Insulin sensitivity, Intravenous glucose tolerance test, Transition period.

1. Introduction

Insulin plays a key role in the nutrient partitioning processes that take place to support lactation in dairy cattle. Cows undergo a period of decreased insulin sensitivity (IS) before and after calving to support fetal glucose needs and to prioritize the insulin-independent uptake of glucose by the mammary gland [1,2]. A dysregulated insulin function during the transition period has been related to several pathological processes in cattle [3,4].

Previous studies investigated the effect of various nutritional strategies [5,6] or administration of different substances [7-11] on IS of the peripheral tissues in dairy cattle. However, the results of those studies are difficult to compare due to the different methods employed to assess IS. The gold standard method for assessing peripheral IS is the hyperinsulinemic euglycemic clamp test [12]. This test is laborious and expensive and, therefore, the intravenous glucose tolerance test (IVGTT) is frequently used to assess IS given its good agreement with the gold

standard test [13,14]. Both these tests, however, are time-consuming and invasive procedures and therefore are not suitable for use under field conditions or on a larger scale in epidemiological investigations [15]. In human medicine, simple and cheap surrogate indices have been developed to assess IS in patients with diabetes that can also be used in large-scale studies. Their intended purpose is to predict IS in the peripheral tissues based on a single blood sample after an overnight fast. Some of these indices have already been applied in studies on dairy cows [4,16-19], but their use has not yet been fully validated. Some studies reported these indices as useful tools to identify lactating cows with disturbed insulin function [17]. Others, however, showed no correlation between different surrogate indices and results from the hyperinsulinemic euglycemic clamp test or IVGTT at the various stages of the transition period [6,13]. Hence, the aim of the present study was to compare IS in dairy cows at the onset of lactation as measured by the IVGTT or through the calculated surrogate indices for IS.

2. Materials and methods

The protocols of this study were approved by the Bioethical Committee of the University of Santiago de Compostela (Spain), and the animals were enrolled with owner consent.

2.1 Animals

Data from the 10 cows of a previous study [7] were used. Selection criteria included: parity (entering their 2nd to 5th lactation), milk production in the preceding lactation (9000 to 9500 kg), body condition score (3 to 3.5, on a 1 [lean] to 5 [obese] scale as previously described [20]), and proximity in their expected calving date. Animals were kept in a free-stall barn with concrete stalls and fed a total mixed ration (Supplementary Table 1), delivered once daily at 9:00 AM.

2.2 Intravenous glucose tolerance test

A detailed description of the IVGTT protocol is presented in the previous study by Abuelo et al. [7]. Briefly, animals were subjected to IVGTT at 3:00 PM between days 3 to 7 after calving. Cows were restrained in feedbunk headlocks and feed was removed from their access. Subsequently, a 14-gauge catheter was inserted in one of the jugular veins and cows were allowed to rest for 15 min before blood sampling started. Blood samples were collected at -10, -5, 5, 10, 20, 30, 45, 60 and 90 min after the infusion of 0.25 g/kg BW of glucose (GlucosaVet 40 g/100 mL,

B. Braun VetCare SA, Barcelona, Spain). The infusion of glucose was completed in 3 to 4 min. After infusion, the catheters were flushed with 10 mL of sterile saline (FisioVet solucion para perfusion, B. Braun VetCare SA, Barcelona, Spain) and the first 5 mL of blood discarded from the first collection. Samples were collected into tubes without anticoagulant (BD Vacutainer; Becton, Dickinson and Company, Plymouth, UK) and tubes containing fluoride heparin (2 mL Glucose Fluoride, Sarsted AG & Co, Nuremberg, Germany).

Values from both baseline samples (-10 and -5 min samples) of each IVGTT were averaged to generate a single baseline value, as previously described [6,9]. The areas under the curve (AUCs) of glucose, insulin, nonesterified fatty acids (NEFA), and β-hydroxybutyrate (BHBA) were computed with the trapezoid method [21] during the 90 min following infusion. Peak and nadir concentrations of these analytes were also determined. Elimination rates and times to reach half-maximal ($T_{1/2}$) and basal (T_{basal}) concentrations for glucose, insulin, NEFA, and BHBA were computed with the following formulas, as previously described by Pires et al. [22]:

$$Elimination\ rate = [(\ln[t_a] - \ln[t_b])/(t_b - t_a)] \times 100$$

$$T_{1/2} = [\ln(2)/Elimination\ rate] \times 100$$

$$T_{basal} = [(\ln[t_a] - \ln[t_b])/Elimination\ rate] \times 100$$

In these formulas, $[t_a]$ is the concentration of the metabolite at time a (t_a) and $[t_b]$ is the concentration of metabolite at time b (t_b).

2.3 Laboratory analysis

Samples were placed on crushed ice and transported to the laboratory, where they were centrifuged at 2000 × *g* for 20 min within 2 h after collection and the supernatant serum or plasma was collected, aliquoted and stored at –80°C pending analysis within 3 mo of collection. Plasma was analyzed for glucose concentration (Glucose-Hexokinase Gernon, RAL Tecnica para el Laboratorio, Barcelona, Spain), whereas serum was used to measure the concentration of nonesterified fatty acids (NEFA H(2) R1+R2 Set, Wako Chemicals GmbH, Neuss, Germany) and β-hydroxybutyrate (BHB, Biochemical enterprise, Milan, Italy). These analytical determinations were performed in duplicate on a biochemistry autoanalyzer (CST-240, DIRUI Industrial Co., Ltd, Changchun, China).

Duplicated serum samples also were analyzed for insulin using a bovine-specific ELISA kit (Bovine Insulin ELISA, Catalog Num. 10–1201-01, Mercodia, Uppsala, Sweden). Conversion of insulin concentration from gravimetric units to international units was done as described by Abuelo et al. [23]. The intra-assay coefficients of variation for all the determinations were below 4.8%, and all samples were analyzed in the same run.

2.4 Surrogate indices for insulin sensitivity

The surrogate indices were calculated using the average of the concentrations of glucose, insulin, NEFA and BHBA in the IVGTT basal samples (-10 and -5 min). The homeostasis model assessment (HOMA), its logarithmic (log_{10}HOMA) and reciprocal score ($HOMA^{-1}$), the quantitative insulin sensitivity check index (QUICKI), the revised quantitative insulin sensitivity check index (RQUICKI), and the revised quantitative insulin sensitivity check index including BHBA (RQUICKI-BHBA) were calculated following the equations in Table 1.

2.5 Statistical analyses

All statistical analyses were performed using SPSS software (SPSS v.20 for Windows, IBM, Chicago, IL, USA). Non-parametric statistical analyses were performed due to the limited sample size (n=10). Hence, the relationships between the different surrogate indexes and the IVGTT parameters were studied statistically with the Spearman's rank correlation coefficient. Clustering of data based on the different treatment groups of the study where the data originated was assessed visually in scatter plots. Statistical significance was declared at $P < 0.05$, and a strong correlation was considered when the absolute value of the correlation coefficient was > 0.50.

3. Results and Discussion

The quartile distribution of the different IVGTT parameters and surrogate indices is shown in Table 2. The guidelines for method comparison studies recommend the visualization of differences using Bland-Altman plots and calculation of the concordance correlation coefficients [24,25]. This requires, however, that the results of both methods are given in identical units. The values derived from the IVGTT and the surrogate indices have different units and, therefore, the direction and strength of the relationships among these parameters were assessed through correlation analysis (Table 3) as has been done in previous human and cattle research [6,13,26].

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The AUCs, elimination rates, and half-lives derived from the IVGTT are usually considered measures of IS. Decreased AUCs, decreased half-life, and increased elimination rates of glucose are thought to involve increased IS [27]. Conversely, high HOMA values, and therefore also high $\log_{10}$(HOMA) and low HOMA^{-1} values, reflect an increase in the concentration of glucose, insulin or both, suggesting in humans a lower peripheral tissue IS. Conversely, high QUICKI, RQUICKI, and RQUICKI-BHBA values reflect lower concentrations of glucose, insulin, and NEFA and BHBA, when applicable, and hence suggest higher IS. From the correlation analysis among AUCs of the variables studied and the surrogate indices calculated, only a significant negative relationship between the $AUC_{insulin}$ and the RQUICKI-BHBA was found (Table 3; r = -0.738, P = 0.023). The direction and strength of the correlation suggest that results from the RQUICKI-BHBA might be in good agreement with the $AUC_{insulin}$. However, $AUC_{insulin}$ on its own is not indicative of IS because cows in early lactation do not release much insulin in response to exogenous glucose [28] due to energy balance being a strong regulator of insulin release during IVGTT [14]. Also, in cases of altered IS other variables from the IVGTT related to glucose, insulin, and NEFA are also altered [6,7,29]. Nevertheless, the RQUICKI-BHBA did not show any other significant correlation with the different variables studied and hence its utility to reflect changes in IS is questionable.

The HOMA, its logarithmic and reciprocal transformations, and the QUICKI indices showed a significant correlation with some of the studied IVGTT variables related to the lipolysis and ketogenesis responses to the glucose infusion (Table 3). These relationships alone, however, do not reflect the state of IS of the animals, but just its stationary metabolic status. For example, the correlation between these indices and the basal concentration of BHBA does not reflect any changes in BHBA concentration in response to the glucose infusion but just relates to the availability of energy substrates prior to the IVGTT. Glucose is preferred over BHBA as energy fuel by cells. Thus, in cows with higher serum concentration of glucose, the production of ketone bodies is usually decreased [30], contributing to a lower basal BHBA concentration. Hence the correlation between HOMA-derived and QUICKI indices with the basal concentration of BHBA.

Peak and nadir NEFA concentrations also showed a significant correlation with HOMA-derived and QUICKI surrogate indices. However, these correlations could be explained by the concentration of energy biomarkers before the infusion. Cows with high HOMA values have high basal concentrations of glucose and insulin. Insulin

inhibits lipolysis, and therefore the maximal and minimal concentration of NEFA should be lower, justifying the negative correlation observed. Interestingly, these correlations were not present with either the RQUICKI or RQUICKI-BHBA indices, which include the basal concentration of NEFA. These two indices were developed by incorporating into their formulas the serum concentration of NEFA, and NEFA and BHB, respectively, to more accurately estimate the IS [16,26] and are therefore considered more robust for the assessment of IS.

Considering the lack of significant correlations between the surrogate indices and the different variables of the IVGTT indicative of IS, particularly with regards to glucose and insulin metabolism, the surrogate indices studied should not be considered suitable to assess the sensitivity of the peripheral tissues to insulin in dairy cows at the onset of lactation. These findings are aligned with the results obtained by De Koster et al. [13] and Mann et al. [6] in dry cows and throughout the transition period, respectively.

The use of the IVGTT to assess IS implies normal insulin secretion after glucose administration and assumes similar insulin secretion among animals, which may not always be the case [2]. Indeed, De Koster et al. [31] have recently reported the limitations of the IVGTT to estimate the changes in IS in dairy heifers from gestation to lactation due to the differences in glucose metabolism. The IVGTT, however still, is considered a good method for assessing IS in cattle given its practicality and agreement with the hyperinsulinemic euglycemic clamp, the gold standard test [13,14]. De Koster et al. [13] also proposed the assessment of the IVGTT results with the minimal mathematical model proposed by Bergman et al. [32] as a good indicator of IS in cattle. This requires a specific proprietary software that was not available to us and therefore not employed in the current study. However, the same researchers also highlighted that the AUCs derived from the IVGTT are a reliable method for assessing IS, based on the agreement with the parameters of the hyperinsulinemic euglycemic clamp in dried-off dairy cattle. The time elapsed between vein cauterization and start of the IVGTT was slightly shorter than other studies, 30 min in the study by Holtenius et al. [5] vs. 15 min in this study. It could be possible that during the stress associated with restraining and catheterization could result in the release of cortisol and interfere with glucose metabolism. However, in trials previous to conducting this experiment we found that the concentrations of glucose and insulin were stable from minute 10 after insertion of the catheter (data not shown).

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Another study limitation is the need to use correlation analysis to assess agreement between two variables, as this is less appropriate than investigating differences between pairs of observations [33], and more easily misinterpreted, particularly in studies with a limited sample size like this one. However, some previous studies using a similar [13] or larger [6] sample size came to similar findings. Additionally, when the data set comprise distinct subgroups, clustering of data can result in an inflated correlation coefficient [33]. However, no obvious clustering patterns were identified, and the absolute value of the majority of correlation coefficients was < 0.50.

4. Conclusions

The surrogate indices for IS studied (HOMA, Log_{10}HOMA, $HOMA^{-1}$, QUICKI, RQUICKI and RQUICKI-BHBA) are not associated with parameters of IS derived from the IVGTT in early lactation dairy cows. Hence, these indices derived from human-based research do not seem suitable to assess the sensitivity of the peripheral tissues to insulin in dairy cows at the onset of lactation. Researchers should, therefore, still rely on more sophisticated and time-consuming methods, such as the IVGTT or the hyperinsulinemic euglycemic clamp test, to accurately assess IS in these animals.

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Conflict of interest: none

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1 ***Table 1*** Calculation of surrogates indices to assess insulin sensitivity.

Name	Equation
Homeostatic model assessment (HOMA)	HOMA = glucose (mmol L^{-1}) × insulin (µIU mL^{-1})
Log-transformation of HOMA	Log_{10} (HOMA) = log_{10} (glucose(mmol L^{-1}) × insulin (µIU mL^{-1}))
Reciprocal score of HOMA	$HOMA^{-1}$ = 1 / (glucose (mmol L^{-1}) × insulin (µIU mL^{-1}))
Quantitative insulin sensitivity check index (QUICKI)	QUICKI = 1 / [log_{10}(glucose (mg dL^{-1})) + log_{10}(insulin (µIU mL^{-1}))]
Revised quantitative insulin sensitivity check index (RQUICKI)	RQUICKI = 1 / [log_{10}(glucose (mg dL^{-1})) + log_{10}(insulin (µIU mL^{-1})) + log_{10}(NEFA (mmol L^{-1}))]
Revised quantitative insulin sensitivity check index including BHBA (RQUICKI-BHBA)	RQUICKI-BHBA = 1 / [log_{10}(glucose (mg dL^{-1})) + log_{10}(insulin (µIU mL^{-1})) + log_{10}(NEFA (mmol L^{-1})) + log_{10}(BHBA (mmol L^{-1}))]

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Table 2. Quartile distribution of the IVGTT parameters and the surrogate indices

	Q1[a]	Q2	Q3
Area under the curve			
Glucose (mmol/L × 90 min)	317.9	434.1	565.6
Insulin (µg/L × 90 min)	72.1	102.5	109.0
NEFA (mmol/L × 90 min)	-40.5	-22.0	-12.7
BHBA (mmol/L × 90 min)	-35.0	-18.3	-8.5
Basal concentration			
Glucose (mmol/L)	2.65	3.33	4.03
Insulin (µg/L)	0.14	0.21	0.53
NEFA (mmol/L)	0.34	0.63	0.82
BHBA (mmol/L)	0.99	1.10	1.94
Peak concentration			
Glucose (mmol/L)	19.54	21.65	28.15
Insulin (µg/L)	1.72	3.30	3.49
NEFA (mmol/L)	0.38	0.49	0.77
BHBA (mmol/L)	1.05	1.23	2.56
Nadir concentration			
Glucose (mmol/L)	3.84	4.34	7.35
Insulin (µg/L)	0.21	0.29	0.84
NEFA (mmol/L)	0.14	0.19	0.34
BHBA (mmol/L)	0.72	0.870	1.37
Elimination rate (%/min)			
Glucose	1.67	2.12	2.70
Insulin	1.39	2.93	3.46
NEFA	1.51	2.39	2.99
BHBA	0.44	0.66	0.97
Time to reach half-maximal concentration (min)			
Glucose	24.8	31.4	41.1
Insulin	20.1	23.7	50.9
NEFA	23.9	29.3	43.2
BHBA	71.8	114.9	168.6
Time to reach basal concentration (min)			
Glucose	55.0	85.0	85.0
Insulin	77.5	85.0	85.0
NEFA	66.1	77.1	112.4
BHBA	278.3	413.9	565.6
Surrogate indices			
HOMA	6.64	16.81	32.24
Log_{10} (HOMA)	0.82	1.23	1.49
$HOMA^{-1}$	0.67	0.82	1.23
QUICKI	0.37	0.40	0.48
RQUICKI	0.43	0.49	0.52
RQUICKI-BHBA	0.41	0.42	0.49

[a] The first quartile (Q1) is the middle number between the smallest number and the median of the data set. The second quartile (Q2) is the median of the data. The third quartile (Q3) is the middle value between the median and the highest value of the data set.

NEFA = Nonesterified fatty acids; BHBA = beta-hydroxybutyrate; HOMA = Homeostatic model assessment; QUICKI = Quantitative insulin sensitivity check index; RQUICKI = Revised quantitative insulin sensitivity check index; RQUICKI-BHBA = Revised quantitative insulin sensitivity check index including β-hydroxybutyrate.

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Table 3. Correlation between the surrogate indices and the different parameters of the intravenous glucose tolerance test.

	Surrogate indices			
	HOMA[a]	QUICKI	RQUICKI	RQUICKI-BHBA
Area under the curve (90 min)				
Glucose	-0.12^{NS}	0.12^{NS}	-0.09^{NS}	-0.33^{NS}
Insulin	0.43^{NS}	-0.43^{NS}	-0.46^{NS}	-0.74*
NEFA	0.22^{NS}	-0.22^{NS}	0.36^{NS}	0.32^{NS}
BHBA	0.33^{NS}	-0.33^{NS}	-0.30^{NS}	0.33^{NS}
Basal concentration				
Glucose	-	-	-	-
Insulin	-	-	-	-
NEFA	-0.43^{NS}	0.43^{NS}	-	-
BHBA	-0.72*	0.72*	0.35^{NS}	-
Peak concentration				
Glucose	-0.27^{NS}	0.27^{NS}	0.19^{NS}	-0.18^{NS}
Insulin	0.42^{NS}	-0.42^{NS}	-0.15^{NS}	-0.16^{NS}
NEFA	-0.80**	0.80**	0.06^{NS}	-0.13^{NS}
BHBA	-0.43^{NS}	0.43^{NS}	0.38^{NS}	-0.23^{NS}
Nadir concentration				
Glucose	0.08^{NS}	-0.08^{NS}	-0.16^{NS}	0.10^{NS}
Insulin	0.27^{NS}	-0.27^{NS}	-0.48^{NS}	-0.44^{NS}
NEFA	-0.82**	0.82**	0.07^{NS}	-0.20^{NS}
BHBA	-0.40^{NS}	0.40^{NS}	0.38^{NS}	-0.23^{NS}
Elimination rate (%/min)				
Glucose	0.05^{NS}	-0.05^{NS}	0.32^{NS}	0.02^{NS}
Insulin	-0.25^{NS}	0.25^{NS}	0.50^{NS}	0.41^{NS}
NEFA	0.31^{NS}	-0.31^{NS}	-0.21^{NS}	0.07^{NS}
BHBA	-0.67*	0.67*	0.44^{NS}	0.27^{NS}
Time to reach half-maximal concentration				
Glucose	-0.14^{NS}	0.14^{NS}	-0.19^{NS}	0.00^{NS}
Insulin	0.25^{NS}	-0.25^{NS}	-0.50^{NS}	-0.41^{NS}
NEFA	-0.24^{NS}	0.24^{NS}	0.20^{NS}	-0.26^{NS}
BHBA	0.45^{NS}	-0.45^{NS}	-0.25^{NS}	-0.30^{NS}
Time to reach basal concentration				
Glucose	-0.45^{NS}	0.45^{NS}	0.04^{NS}	0.41^{NS}
Insulin	0.23^{NS}	-0.23^{NS}	-0.17^{NS}	-0.16^{NS}
NEFA	-0.11^{NS}	0.11^{NS}	0.44^{NS}	0.14^{NS}
BHBA	0.72*	-0.72*	-0.37^{NS}	-0.31^{NS}

*The table shows the Spearman's rank correlation coefficient (r). * = P <0.05; ** = P<0.01; ^{NS} = not significant; NEFA = Nonesterified fatty acids; BHBA = beta-hydroxybutyrate; - = Not calculated as it is used to calculate the surrogate index. [a] Correlation coefficients for the logarithmic and inverse transformation of HOMA are identical to those of HOMA.*