Comparison of measures of insulin sensitivity in early-lactation dairy goats

F. Zamuner,1* A. W. N. Cameron,2 B. J. Leury,1 and K. DiGiacomo1

Graphical Abstract

Summary
This experiment aimed to examine the association between surrogate indices of insulin resistance (IR)—namely, the homeostasis model of IR (HOMA-IR), quantitative insulin sensitivity check index (QUICKI), and revised quantitative insulin sensitivity check index (RQUICKI)—and measures of IR obtained from an intravenous glucose tolerance test (IVGTT) performed in early-lactation dairy goats. Despite a moderately strong correlation between surrogate indices of IR and insulin area under the curve, we found no significant relationship between surrogate indices and measures of reduced insulin sensitivity, such as glucose clearance rate, glucose area under the curve, and insulin sensitivity index. Thus, our results suggest that surrogate indices of IR are not suitable for assessing the insulin sensitivity of peripheral tissue in early-lactation goats.

Highlights
- Surrogate indices of IR and measures of insulin sensitivity derived from the IVGTT were not correlated.
- Surrogate indices were moderately correlated with measures of insulin secretion.
- Surrogate indices of IR are not indicated for assessing peripheral tissue insulin sensitivity in early-lactation goats.
Comparison of measures of insulin sensitivity in early-lactation dairy goats

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Abstract: This experiment aimed to investigate the correlations between surrogate indices of insulin resistance (IR)—namely, the homeostasis model of IR, the quantitative insulin sensitivity check index, and the revised quantitative insulin sensitivity check index—and measures of IR obtained from an intravenous glucose tolerance test (IVGTT) performed in early-lactation dairy goats. Saanen goats (n = 26) with varying levels of milk production (1.7–4.8 kg/d) were selected and underwent an IVGTT on 43 ± 0.7 d postpartum (mean ± standard deviation). Data from the IVGTT were fitted in the minimal model to calculate parameters of glucose–insulin dynamics such as insulin sensitivity index and acute insulin response to glucose. Surrogate indices were computed using the average of the IVGTT basal concentrations of insulin and glucose clearance rate. Correlation analysis revealed no relationship between surrogate indices of IR and measures of IR derived from the IVGTT (e.g., insulin sensitivity index, glucose clearance rate, glucose area under the curve). Therefore, our results suggest that surrogate indices of IR are not suitable for assessing the insulin sensitivity of peripheral tissue in early-lactation goats.
presented in Zamuner et al. (2020a). Briefly, goats were kept in individual stalls (15 m²) from 11 to 45 ± 4.2 d postpartum (mean ± SD) and ad libitum fed a TMR once daily at around 0900 h.

On the morning of d 42 (wk 6) after an overnight fast, a 14-gauge, 3.25-inch angiocath catheter (BD) was inserted into the jugular vein. A 22-cm plastic catheter extension with a Luer lock (Heidelberg extension tubing; B. Braun) prefilled with heparinized saline (50 U/L) was secured to the catheter. During blood sampling procedures, the catheter was flushed with heparinized saline (25 U/L) immediately after every blood sample collection. On d 43 (after overnight fasting) a 50% glucose solution was administered intravenously at 0.3 g of glucose/kg of BW, and blood samples were collected at −30, −15, −1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 60, 90, 120, 150, 180, 210, 220, and 240 min relative to glucose infusion. All blood samples were collected into vacuum tubes (10 mL) coated with lithium heparin (BD), immediately placed on ice, and centrifuged (1,250 × g) for 12 min immediately after collection. Isolated plasma was stored at −20°C until analysis.

Plasma fatty acids concentrations were measured using a commercially available kit (NEFA-C ACS-ACOD method, modified per the methods of Johnson and Peters, 1993; Wako Pure Chemical Industries Ltd.). Plasma glucose concentrations were measured using a commercially available kit (Infinity Glucose Oxidase Liquid, Thermo Fisher Scientific). Plasma insulin concentrations were measured using a RIA kit (Porcine Insulin cat. no. PI-12K, Millipore Corp.) validated in goats by Maia-Nogueira (2015). Assay sensitivity (limit of detection) ranged between 0.4 and 0.5 mU/L for insulin. Every sample was assayed in duplicate. Intra- and interassay coefficients of variation, respectively, were <7.0% and <3.5% for glucose, <6.0% and <3.0% for fatty acids, and <10% and <4.1% for insulin.

Basal concentrations for insulin, glucose, and fatty acids were calculated as the mean concentration of the 3 blood samples taken before the glucose infusion. Plasma insulin, glucose, and fatty acid responses were analyzed for the area under the curve (AUC) using a linear trapezoidal summation between successive pairs of metabolite concentrations after correcting for baseline concentrations. Clearance rates (CR), time to reach half-life (T1/2), and basal concentrations (Tb) were calculated per Pires et al. (2007) and surrogate indices were calculated per Singh and Saxena (2010) using the following equations:

\[
CR_{glucose} = \left[ \ln (mM \text{ at } 2 \text{ min}) - \ln (mM \text{ at } 60 \text{ min})/ (60 - 2 \text{ min} \times 100 \right.
\]

\[
CR_{insulin} = \left[ \ln (mIU/L \text{ at } 20 \text{ min}) - \ln (mIU/L \text{ at } 60 \text{ min})/ (60 - 20 \text{ min} \times 100 \right.
\]

\[
T_{1/2} = \left[ \ln (2)/CR \times 100 \right.
\]

\[
T_b = \left[ (\ln (2 \text{ min}) - \ln (240 \text{ min}))/CR \right] \times 100
\]

HOMA-IR = \left[ \text{basal glucose (mM} \times \text{basal insulin (mIU/L)})/22.5
\]

\[
\text{QUICKI} = 1/[\ln \text{ glucose (mg/dL)} + \ln \text{ insulin (mIU/L)}]
\]

Descriptive statistics for indices derived from the IVGTT and from the minimal model, surrogate indices, and performance traits are presented in Table 1. Basal concentrations and AUC for glucose, insulin, and fatty acids were within the range reported in healthy dairy goats (Cai et al., 2018). The large variation of milk production (1.7–4.8 kg/d) and the potential variation observed in metabolism between humans and ruminants. For instance, in ruminants, it is impossible to reach a basal steady-state condition without going through prolonged starvation, which in turn would cause changes to insulin, glucose, and fatty acids concentrations unrelated to the state of IR of the animal (De Koster et al., 2017).
Additionally, some common features of the periparturient period in ruminants are negative energy balance, decreased insulin and glucose concentration, and increased fatty acids concentration (De Koster and Opsomer, 2013). Such physiological differences make it difficult to extrapolate to ruminants the interpretation of measures of IR in human medicine (De Koster et al., 2016; Cincović et al., 2017; Hasegawa et al., 2011; Cincović et al., 2014). Apparently, the same principle can be applied to comparisons between goats and cows. Therefore, due to the lack of information on the differences in the magnitude of changes in the fatty acids:insulin ratio between postpartum and antepartum animals, which might have a large effect on absolute RQUICKI values. For instance, Marinković et al. (2019) and Cincović et al. (2014) reported a significant decrease in insulin concentrations (~35%) but an approximate 250% increase in fatty acids concentrations in postpartum cows. Conversely, in Zamuner et al. (2020b), we observed that the increase in fatty acids concentration in postpartum goats (+188%) was accompanied by a more pronounced decrease in insulin concentration (~312%) compared with antepartum goats. Similarly, in Zamuner et al. (2020a), we observed that greater fatty acids concentration in high-yielding goats (+20%) was accompanied by a much lower insulin concentration (−130%) compared with antepartum goats. Schoenberg and Overton (2011) pointed out the weaknesses of using RQUICKI to measure IR in ruminants, suggesting that interpretation of results between cows of different metabolic status or stage of lactation should be done with caution. Apparently, the same principle can be applied to comparisons between goats and cows. Therefore, due to the lack of information on dairy goats, it is rather difficult to compare our results with those reported in the literature. Further research is needed to determine the potential use of surrogate indices of IR to measure IR in dairy goats.

### Table 1. Descriptive statistics of measures of insulin sensitivity derived from the intravenous glucose tolerance test, surrogate indices for insulin resistance, and performance traits in early-lactation dairy goats (n = 26)

| Variable1 | Mean | SEM | SD | CV |
|-----------|------|-----|----|----|
| **Glucose** | | | | |
| Basal (mM) | 3.2 | 0.10 | 0.49 | 15 |
| CR0–40 (%/min) | 2.3 | 0.10 | 0.49 | 21 |
| T1/2 (min) | 31 | 1.2 | 6.1 | 20 |
| T90 (min) | 78 | 3.5 | 17.6 | 23 |
| AUC (mM/min) | 359 | 23.3 | 118.6 | 33 |
| **Insulin** | | | | |
| Basal (mU/L) | 9.3 | 1.43 | 7.31 | 79 |
| CR0–40 (%/min) | 2.3 | 0.27 | 1.4 | 59 |
| T1/2 (min) | 62 | 5.0 | 25.3 | 41 |
| AUC (mU/L per min) | 5,066 | 611.0 | 3,170 | 62 |
| **Fatty acids** | | | | |
| Basal (mM) | 0.5 | 0.07 | 0.37 | 69 |
| AUC (mM/min) | −9.9 | 5.63 | 28.7 | −290 |
| HOMA-IR | 1.4 | 0.24 | 1.23 | 86 |
| QUICKI | 0.42 | 0.025 | 0.127 | 30 |
| RQUICKI | 0.47 | 0.020 | 0.103 | 22 |
| Si (mU/L per min) | 3.9 | 0.78 | 3.90 | 99 |
| Sg ×10^3 (mM/min) | 16.5 | 0.97 | 4.84 | 29 |
| AIR (mU/L per min) | 455 | 54.73 | 186.5 | 52 |
| ECM (kg/d) | 3.2 | 0.18 | 0.89 | 28 |
| DMI (%) | 3.1 | 0.14 | 0.71 | 23 |
| BW (kg) | 67 | 6.1 | 7.9 | 12 |
| BCS | 2.3 | 0.10 | 0.53 | 22 |

1CR = clearance rate; T1/2 and T90 = time to reach half-life and basal concentration after the glucose infusion, respectively; AUC = area under the response curve during the first 120 min of the intravenous glucose tolerance test; HOMA-IR = homeostasis model of insulin resistance; QUICKI = quantitative insulin sensitivity check index; RQUICKI = revised quantitative insulin sensitivity check index.

### Table 2. Spearman rho correlations between measures of insulin sensitivity derived from the intravenous glucose tolerance test, surrogate indices of insulin resistance, and performance traits in early-lactation dairy goats (n = 26)

| Item2 | HOMA-IR | QUICKI | RQUICKI |
|-------|---------|--------|---------|
| **Glucose** | | | |
| Basal (mM) | 0.76*** | −0.76*** | −0.60** |
| CR0–40 (%/min) | 0.06** | −0.06** | 0.22** |
| T1/2 (min) | −0.33** | 0.33** | 0.11** |
| T90 (min) | −0.15** | 0.15** | −0.19** |
| AUC (mM/min) | −0.13** | 0.13** | −0.14** |
| **Insulin** | | | |
| Basal (mU/L) | 0.99*** | −0.99*** | −0.89*** |
| CR0–40 (%/min) | −0.26** | 0.26** | 0.33† |
| T1/2 (min) | −0.21** | 0.21** | 0.32** |
| AUC (mU/L per min) | 0.53** | −0.53** | −0.56** |
| **Fatty acids** | | | |
| Basal (mM) | −0.46* | 0.46* | 0.09** |
| AUC (mM/min) | 0.36† | −0.36† | −0.08** |
| Si (mU/L per min) | −0.18NS | 0.18NS | 0.25** |
| Sg ×10^3 (mM/min) | 0.05NS | −0.05NS | 0.12** |
| AIR (mU/L per min) | 0.55** | −0.55** | −0.51** |
| ECM (kg) | −0.52** | 0.52** | 0.56** |
| DMI (% of BW) | −0.54** | 0.54** | 0.60** |
| BCS | 0.51** | −0.51** | −0.48** |

1HOMA-IR = homeostasis model of insulin resistance; QUICKI = quantitative insulin sensitivity check index; RQUICKI = revised quantitative insulin sensitivity check index.

2CR = clearance rate; T1/2 and T90 = time to reach half-life and basal concentration after the glucose infusion, respectively; AUC = area under the response curve during the first 120 min of the intravenous glucose tolerance test; Si = insulin sensitivity; Sg = glucose effectiveness; AIR = acute insulin response to glucose.

*P < 0.05; **P < 0.01; ***P < 0.001; †P < 0.1; NS > 0.1.
In humans, the pancreas compensates for reduced insulin action in peripheral tissues by upregulating insulin secretion (De Koster and Opsomer, 2013). In the present study, we observed a negative correlation between SI and insulin AUC (r = -0.59, P = 0.002) and between SI and insulin AIR (r = -0.45, P = 0.023). At first glance, the direction and strength of these correlations could be interpreted as compensatory hypersecretion of insulin in response to lower insulin sensitivity. However, these results should be interpreted with caution because in Zamuner et al. (2020a) we found no difference in peripheral tissue response to insulin between goats of different basal insulin levels (5.6 vs. 12.9 mU/L, P = 0.008). Therefore, the observed large interindividual variations in insulin production and secretion could be attributed to genetic differences, as has been demonstrated in humans (Hansen et al., 2020), or to differences in milk production and energy status (Zamuner et al., 2020a). Nevertheless, the reasons for the present findings remain speculative.

Several authors have reported a negative association between fatty acids concentration and pancreatic insulin secretion in dairy cows (De Koster and Opsomer, 2013; Cinčović et al., 2018; Hasegawa et al., 2019). For the influence of fatty acids on glucose–insulin kinetics (or vice versa) in dairy goats, our results are somewhat ambiguous. In the present study, the increased basal concentration of fatty acids was associated with reduced basal insulin, reduced glucose CR, and increased glucose AUC, T_{1/2}, and T_0 (r = -0.47, -0.61, 0.65, 0.48, and 0.61, respectively; P < 0.01), suggesting that increased lipid mobilization was associated with increasing IR. Nevertheless, we found no significant correlation between basal fatty acids and insulin AUC or between basal fatty acids and any minimal model–derived measures of IR (SI, Sg, AIR). Therefore, more detailed investigations are needed to determine the role, if any, of lipid mobilization in glucose–insulin kinetics in dairy goats.

Considering the lack of significant correlations between the surrogate indices of IR and measures of insulin sensitivity derived from the IVGTT and the minimal model, we suggest that the studied surrogate indices of IR are not suitable for assessing insulin resistance of peripheral tissue in early-lactation goats. Nevertheless, given the relatively small set of animals used in the present study and the limited literature on insulin production, secretion, and sensitivity in dairy goats, further research is needed to confirm, or to expand on, the potential use of surrogate indices of IR to measure differences in IR, or in energy status, in early-lactation goats. Moreover, RQUICKI values were strongly correlated with basal insulin and moderately correlated with insulin AUC and AIR, but no significant correlation was found between RQUICKI and basal fatty acids or fatty acids AUC.

References

Boston, R. C., D. Stefanovski, P. J. Moate, A. E. Sumner, R. M. Watanabe, and R. N. Bergman. 2003. MINMOD Millennium: A computer program to calculate glucose effectiveness and insulin sensitivity from the frequently sampled intravenous glucose tolerance test. Diabetes Technol. Ther. 5:1003–1015. https://doi.org/10.1089/15209150322641060.

Cai, J., F. Q. Zhao, J. Liu, and D. Wang. 2018. Local mammary glucose supply regulates availability and intracellular metabolic pathways of glucose in the mammary gland of lactating dairy goats under malnutrition of energy. Front. Physiol. 9:1467. https://doi.org/10.3389/fphys.2018.01467.

Cinčović, M., B. Belić, R. Djkovic, B. Toholj, and T. Hristovska. 2014. Insulin resistance in cows during dry period and early lactation. Contemp. Agric. 63:98–105.

Cinčović, M., D. Kirovski, I. Vujanac, B. Belić, and R. Djković. 2017. Relationship between the indexes of insulin resistance and metabolic status in dairy cows during early lactation. Acta Vet. (Beograd) 67:57–70. https://doi.org/10.1515/acve-2017-0006.

Cinčović, M. R., R. Djkovic, B. Belić, I. Lakić, N. Stojanac, O. Stevančević, and N. Staničkov. 2018. Insulin resistance in cows during the periparturient period. Acta Agr. Serb. 23:233–245. https://doi.org/10.5937/AAser184623SC.

De Koster, J., M. Hostens, K. Hermans, W. Van den Broeck, and G. Opsomer. 2016. Validation of different measures of insulin sensitivity of glucose metabolism in dairy cows using the hyperinsulinemic euglycemic clamp test as the gold standard. Domest. Anim. Endocrinol. 57:117–126. https://doi.org/10.1016/j.domaniend.2016.06.004.

De Koster, J., D. and G. Opsomer. 2013. Insulin resistance in dairy cows. Vet. Clin. North Am. Food Anim. Pract. 29:299–322. https://doi.org/10.1016/j.cvfa.2013.04.002.

De Koster, J., M. Van Eevelde, K. Hermans, W. Van Den Broeck, M. Hostens, and G. Opsomer. 2017. Limitations of glucose tolerance tests in the assessment of peripheral tissue insulin sensitivity during pregnancy and lactation in dairy heifers. J. Dairy Sci. 100:2381–2387. https://doi.org/10.3168/jds.2016-11792.

DeFrongo, R. A., J. D. Tobin, and R. Andres. 1979. Glucose clamp technique: A method for quantifying insulin secretion and resistance. Am. J. Physiol. 237:E214–E223. https://doi.org/10.1152/ajpendo.1979.237.3.E214.

Duelmeier, R., I. Fluegge, B. Schwert, and M. Ganter. 2013. Insulin sensitivity during late gestation in ewes affected by pregnancy toxemia and in ewes with high and low susceptibility to this disorder. J. Vet. Intern. Med. 27:359–366. https://doi.org/10.1111/jvim.12035.

Hahn, R. G., S. Ljunggren, F. Larsen, and T. Nyström. 2011. A simple intravenous glucose tolerance test for assessment of insulin sensitivity. Theor. Biol. Med. Model. 8:12. https://doi.org/10.1186/1742-4682-8-12.

Hansen, A. M. B., C. Wium, S. Lee, A. C. Tierney, D. McCarthy, H. M. Roche, C. A. Drevon, K. I. Birkeland, and H. L. Gulseth. 2020. Substantial interindividual variations in insulin secretion and sensitivity across the glucose-metabolic spectrum. Scand. J. Clin. Lab. Invest. 80:282–290. https://doi.org/10.1080/00365513.2020.1730433.

Hasegawa, R., J. Iwasue, T. Takagi, M. Kondo, M. Matsui, and C. Kawashima. 2019. Insulin resistance: Relationship between indices during late gestation in dairy cows and effects on newborn metabolism. Anim. Sci. J. 90:1544–1555. https://doi.org/10.1111/asj.13300.

Holtenius, P., and K. Holtenius. 2007. A model to estimate insulin sensitivity in dairy cows. Acta Vet. Scand. 49:29. https://doi.org/10.1186/1751-0147-49-29.

Johnson, M. M., and J. P. Peters. 1993. An improved method to quantify non-esterified fatty acids in bovine plasma. J. Anim. Sci. 71:753–756. https://doi.org/10.2527/1993.717355x.

Kahn, C. R. 1978. Insulin resistance, insulin insensitivity, and insulin unresponsiveness: A necessary distinction. Metabolism 27(Suppl. 2):1893–1902. https://doi.org/10.1016/S0026-0495(78)8007-9.

Kampmann, U., S. Knorr, J. Fuglsang, and P. Ovesen. 2019. Determinants of maternal insulin resistance during pregnancy: An updated overview. J. Diabetes Res. 2019:5320156. https://doi.org/10.1155/2019/5320156.

Maia-Nogueira, D. 2015. The meat goat industry in Australia: Geographical, seasonal and nutritional influences on reproduction in female goats. PhD Diss. College of Public Health, Medical and Veterinary Sciences, James Cook University, Townsville, Queensland, Australia.

Mann, S., F. L. Yepes, M. Duplessis, J. Wakshtag, T. Overton, B. Cummings, and D. Nydam. 2016. Dry period plane of energy: Effects on glucose tolerance in transition dairy cows. J. Dairy Sci. 99:701–717. https://doi.org/10.3168/jds.2015-9908.

Marinković, M. B., B. Belić, M. R. Cinčović, R. Djković, I. Lakić, N. Stojanac, O. Stevančević, and G. Devecerski. 2019. Relationship between insulin, glucose, non-esterified fatty acid and indices of insulin resistance in obese cows during the dry period and early lactation. Acta Vet. Brno 88:143–155. https://doi.org/10.2754/avb201988020143.

Miao, Z., H. Wu, L. Ren, N. Bu, L. Jiang, H. Yang, J. Zhang, and X. Guo. 2020. Long-term postpartum outcomes of insulin resistance and β-cell function in women with previous gestational diabetes mellitus. Int. J. Endocrinol. 2020:1–7. https://doi.org/10.1155/2020/7417356.

Muniyappa, R., S. Lee, H. Chen, and M. J. Quon. 2008. Current approaches for assessing insulin sensitivity and resistance in vivo: Advantages, limitations,
and appropriate usage. Am. J. Physiol. Endocrinol. Metab. 294:E15–E26. https://doi.org/10.1152/ajpendo.00645.2007.

Opsomer, G., T. Wensing, H. Laevens, M. Coryn, and A. de Kruif. 1999. Insulin resistance: The link between metabolic disorders and cystic ovarian disease in high yielding dairy cows? Anim. Reprod. Sci. 56:211–222. https://doi.org/10.1016/S0378-4320(99)00048-2.

Pires, J. A., A. Souza, and R. Grummer. 2007. Induction of hyperlipidemia by intravenous infusion of tallow emulsion causes insulin resistance in Holstein cows. J. Dairy Sci. 90:2735–2744. https://doi.org/10.3168/jds.2006-759.

Schoenberg, K. M., and T. Overton. 2011. Effects of plane of nutrition and 2,4-thiazolidinedione on insulin responses and adipose tissue gene expression in dairy cattle during late gestation. J. Dairy Sci. 94:6021–6035. https://doi.org/10.3168/jds.2011-4533.

Singh, B., and A. Saxena. 2010. Surrogate markers of insulin resistance: A review. World J. Diabetes 1:36–47. https://doi.org/10.4239/wjd.v1.12.36.

Wilcox, G. 2005. Insulin and insulin resistance. Clin. Biochem. Rev. 26:19–39.

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Notes

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