J. Dairy Sci. 100:1–7
https://doi.org/10.3168/jds.2016-11792

Short communication: Limitations of glucose tolerance tests in the assessment of peripheral tissue insulin sensitivity during pregnancy and lactation in dairy heifers

J. De Koster,*[1] M. Van Eetvelde,* K. Hermans,* W. Van den Broeck,† M. Hostens,* and G. Opsomer*
*Department of Reproduction, Obstetrics and Herd Health, and
†Department of Morphology, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

ABSTRACT

The aim of the present study was to point at the limitations of glucose tolerance tests (GTT) to assess peripheral tissue insulin sensitivity in dairy heifers in different physiological states (pregnancy and lactation). Intravenous GTT were performed in 5 nonpregnant, nonlactating heifers, 5 heifers at the end of pregnancy (12–7 d before calving), and 5 lactating primiparous cows (11–14 d after calving). Glucose and insulin concentrations were determined and area under the curve (AUC) and clearance rate of glucose and insulin were calculated. Additionally, data were analyzed using the minimal model to derive the insulin sensitivity parameter (Si). Basal glucose and insulin concentrations were greater in the nonpregnant, nonlactating heifers. Clearance rate of glucose and Si were lowest, whereas the AUC for glucose was greatest in the nonpregnant, nonlactating heifers. Insulin concentrations during the GTT were greater for the nonpregnant, nonlactating heifers. Results from the GTT in pregnant heifers and lactating primiparous cows are biased by the fact that a large part of the glucose disappearance during an intravenous GTT occurs independently of insulin by the pregnant uterus or the lactating mammary gland. As such, greater AUC of glucose, lower clearance rate of glucose, or lower Si derived from GTT performed in nonpregnant, nonlactating dairy heifers in the present study might indicate decreased peripheral tissue insulin sensitivity of the glucose metabolism or decreased insulin-independent glucose disappearance. Based on the results from a GTT, it is impossible to discriminate between both metabolic pathways. It can be concluded that parameters derived from GTT are not suited to compare peripheral tissue insulin sensitivity of the glucose metabolism between dairy heifers in different physiological states due to the large variation in insulin secretion and the substantial difference in insulin-independent glucose disposal associated with these physiological states.

Key words: glucose tolerance test, pregnancy, lactation, insulin resistance

Short Communication

Glucose tolerance tests (**GTT**) are frequently used to assess insulin sensitivity in dairy cows (Holtenius et al., 2003; Chagas et al., 2009). The plasma glucose profile during a GTT is the reflection of total body glucose metabolism after an intravenous glucose bolus (Ferrannini and Mari, 1998; De Koster and Opsomer, 2013). The total body glucose metabolism can be subdivided into insulin-independent and insulin-dependent glucose metabolism. The insulin-dependent glucose metabolism is influenced by the increase in insulin concentration after an intravenous glucose bolus and stimulates insulin-sensitive tissues (mainly skeletal muscle and, to a lesser extent, adipose tissue) to increase SLC2A4 (solute carrier family 2, facilitated glucose transporter member 4; formerly known as GLUT4) translocation to the plasma membrane followed by a subsequent increase in glucose uptake. The response of insulin-sensitive tissues to insulin determines the insulin resistance of the animal (Kahn, 1978; De Koster and Opsomer, 2013).

The insulin-independent glucose metabolism is not influenced by the increased insulin concentration during the GTT, but is mainly determined by the capacity of an increased glucose concentration to enhance its own disappearance and to inhibit hepatic glucose output (Bergman, 2007; Muniyappa et al., 2008). In nonpregnant, nonlactating animals, the insulin-independent glucose disappearance occurs mainly via glucose transporter 1 (GLUT1) in different tissues and by excretion via the kidney (only small amounts, 2 to 4% of the glucose bolus; Grünberg et al., 2011). In pregnant animals, a large part of the circulating glucose (60 to 70%) is taken up independently of insulin by the pregnant uterus via GLUT1 and GLUT3 (Bell et al.,

Received July 28, 2016.
Accepted November 29, 2016.
[1]Corresponding author: jenne.dekoster@ugent.be

2000; Grünberg et al., 2011). This uptake is even more pronounced in lactating animals, in which the insulin-independent glucose uptake by the udder via GLUT1 increases glucose requirements substantially (De Koster and Opsomer, 2013). In lactating cows, insulin-independent glucose uptake by the udder was estimated to be responsible for 80 to 82% of whole body glucose turnover (Bickerstaffe et al., 1974; Bell and Bauman, 1997; Rose et al., 1997).

The ability of a test to assess the insulin sensitivity of the glucose metabolism is dependent on its ability to differentiate between the effect of insulin to enhance glucose disappearance and all other factors influencing glucose disappearance (De Koster et al., 2016). Several different tests have been used to assess insulin resistance in dairy cows. Surrogate indices for insulin sensitivity (calculated from glucose, insulin, fatty acids, and BHB concentrations in blood) were unreliable to assess insulin resistance in dairy cows at the end of the dry period due to inherent differences in metabolism between humans and ruminants. In humans, insulin resistance is characterized by high insulin or high glucose concentrations, whereas the metabolism of dry and lactating cows is characterized by low glucose and low insulin concentrations (Bloomgarden, 2006; De Koster and Opsomer, 2013; De Koster et al., 2016). In humans, elevated concentrations of triglycerides have been associated with insulin resistance (Bloomgarden, 2006), whereas fatty acids and BHB in dairy cows are reflections of negative energy balance rather than a state of insulin resistance (Ospina et al., 2013; De Koster et al., 2016). Parameters derived from GTT (area under the curve for glucose, insulin sensitivity index derived from the minimal model) were proven to be reliable estimates of insulin resistance in dairy cows at the end of the dry period (De Koster et al., 2016). Dairy cows generally are in a lactating or pregnant state, leading, in large part, to glucose disappearance being insulin-independent (60 to 82%; Rose et al., 1997; De Koster and Opsomer, 2013) and making it difficult to interpret and compare parameters derived from GTT performed in cows in different physiological states (Marett et al., 2015). In the literature, however, multiple examples can be found in which GTT has been used to measure and compare insulin resistance in cows in different physiological states without taking into account the potential difference in insulin-independent glucose disappearance or differences in glucose-induced insulin secretion (Chalmeh et al., 2015; Oliveira et al., 2016). The aim of the present study was to point at the limitations of GTT to assess peripheral tissue insulin sensitivity in dairy heifers in different physiological states (pregnancy and lactation). We hypothesized that the results from GTT would differ depending on the physiological state of the animals, but interpretation of the parameters derived from GTT would be indefinite in terms of insulin resistance.

All experimental procedures were approved by the ethical committee of the Faculty of Veterinary Medicine (EC2015/142 – Ghent University, Belgium). Intravenous GTT were performed in nonpregnant, nonlactating heifers (**NON**, n = 5), pregnant, nonlactating heifers 12 to 7 d before calving (**PREG**, n = 5), and nonpregnant, lactating primiparous cows 11 to 14 d after calving (**LACT**, n = 5). On the day of the GTT, heifers were weighed, BCS was assessed according to the scale of Edmonson et al. (1989), and back fat thickness (**BFT**) was determined as described by Schröder and Staufenbiel (2006). A catheter (Cavafix Certo 338-14G, B. Braun, Instrulife, Oostkamp, Belgium) was placed in the left jugular vein and heifers were allowed to rest for a period of 2 h. Glucose was infused at a dose of 150 mg/kg of BW (Glucose 30%, Eurovet, Verdifarm, Beringen-Paal, Belgium) over a period of 2 to 4 min, after which the catheter was flushed 2 times with 20 mL of saline. Time point 0 was the moment when all the glucose was infused. Blood samples for determination of glucose and insulin were taken at following time points: −15, −5, 0, 2, 4, 6, 8, 10, 12, 15, 18, 20, 23, 26, 30, 35, 40, 45, 50, 60, 90, 120, 150, and 180 min. From 2 h before until the end of the GTT, heifers had access to fresh drinking water but not feed.

Samples for plasma glucose determination were taken in fluoride blood tubes (Vacutest, Novolab, Geraardsbergen, Belgium). Samples for serum insulin determination were taken in gel-coated blood tubes (Vacutest, Novolab). Within 2 h after collection, all blood samples were centrifuged for 20 min (2,460 × *g*, 7°C) and serum and plasma were stored at −20°C until analysis. Plasma glucose concentrations were determined using a colorimetric hexokinase method on a Cobas 6000 analyzer (Roche Diagnostics, Mannheim, Germany), and intra- and interassay coefficient of variation were 0.82 and 1.1%, respectively. Serum insulin concentrations were determined using a bovine-specific commercial ELISA kit (Bovine Insulin ELISA, Catalog nr 10–1201–01, Mercodia, Uppsala, Sweden), and intra- and interassay CV were 2.9 and 10.8%, respectively. Conversion of insulin concentrations from gravimetric units to international units was done as described by Abuelo et al. (2012).

Based on the measured glucose and insulin concentrations during the GTT, different measures of insulin sensitivity were calculated. The clearance rate (**CR**) of glucose between 0 and 30 min and 0 and 60 min and the clearance rate of insulin between 15 and 30 min were