J. Dairy Sci. 100:1–10
https://doi.org/10.3168/jds.2016-12133

Influence of food deprivation on intravenous glucose tolerance test traits in Holstein Friesian heifers

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ABSTRACT

Glucose is essential for numerous cellular functions due to its involvement in energy supply from early development to adulthood. In the lactating cow, glucose demands by the mammary gland significantly increase to support milk production when compared with other tissues. Thus, insufficient blood glucose levels might predispose dairy cows to metabolic disturbances. The intravenous glucose tolerance test (ivGTT) is a suitable tool to characterize glucose metabolism and insulin responses, but results must be reliable and repeatable. One factor influencing ivGTT is food deprivation period, which has been considered as an obligatory requirement before conduction of glucose tolerance studies in monogastric species, whereas it has been permissive in ruminants. The objective of this study was to determine the influence of 5 fasting periods (0, 12, 24, 36, and 48 h) on ivGTT traits and insulin responses in German Holstein heifers. A total of 140 tests were conducted in 28 females aged 12 to 19 mo. Blood samples were collected every 7 min within 1 h. Assessed glucose and insulin parameters included basal serum glucose and insulin concentration, maximum glucose and insulin concentration obtained between min 7 to 21, and concentrations at min 63 (last sampling) relative to glucose administration, glucose area equivalent (GA), glucose area under the curve (GAUC), insulin area equivalent (InsA), insulin area under the curve (InsAUC), and blood glucose half-life time (GHLT). Serum glucose and insulin concentration were measured according to the hexokinase colorimetric method and solid phase radio immunoassay, respectively. The generalized linear mixed model was used to test for significant differences in all glucose traits and insulin responses at different fasting periods. The model used season and weight as confounding variables. Glucose and insulin concentrations at 0, 7 to 21 (maximum concentration), and 63 min were affected by the duration of food deprivation. The GA, InsA, GAUC, InsAUC, and especially GHLT were also affected by fasting period. A positive linear relationship between GHLT and length of food deprivation was found. Significantly higher GA, GAUC, GHLT values, and glucose and insulin concentration at min 63 were obtained at increasing fasting periods. High intraclass correlation coefficient (0.48) was found for GHLT. The results demonstrate that GHLT might be used to reliably characterize an individual's glucose metabolism and the importance of standardizing food deprivation schedules when performing an ivGTT in nongestating Holstein heifers.
Key words: metabolism, glucose tolerance test, glucose half-life time, fasting period

INTRODUCTION

Glucose metabolism has been extensively investigated in cattle due to its essential roles in numerous cellular processes. In dairy cows, balanced glucose metabolism is indispensable to ensure high milk yield, good fertility, and general health. Assessing the ability of an organism and its efficiency to properly secrete insulin and use glucose is commonly performed through an oral or intravenous glucose tolerance test (**ivGTT**) over a fixed period of time (Kaneko, 2008). The values derive from calculations denoting glucose half-life time (**GHLT**), glucose area equivalent (**GA**), and the rate at which the metabolite is cleared from the blood, among others (Freyer et al., 2004). In cattle, ivGTT is commonly used to test for conditions such as insulin resistance (De Koster and Opsomer, 2013) or determine the levels of insulin sensitivity (Abuelo et al., 2016). The test can also predict changes in nonesterified fatty acids (Boston et al., 2008) and determine the effects of certain diets on energy metabolism (Kneeskern et al., 2016). Furthermore, Pieper et al. (2016) found high heritability for some ivGTT traits in young Holstein bulls including GA and GHLT with estimated heritabilities of 0.43 and

Received October 10, 2016.
Accepted May 11, 2017.
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0.40, respectively. Therefore, altered glucose tolerance values could be linked to impaired metabolic responses at early stages, which could become an important selection criterion for animal health in the future. Other studies have proposed that glucose responses could predict the genetic ability for milk production in female calves (Sasaki et al., 2003) and heifers (Fiedorowicz et al., 2008).

Although the use of ivGTT in domestic cattle has generated significant advances in many fields, some factors (e.g., feed deprivation, glucose dose) influencing ivGTT traits have not fully been investigated. In monogastric species, food deprivation is crucial before sampling (Verkest et al., 2010) because high gluconeogenesis rates occur during the fasting phases (reviewed by Aschenbach et al., 2010). Conversely, numerous studies involving domestic cattle have omitted fasting periods, whereas others have implemented either food restriction during the examination or prolonged times. A summary of studies employing different fasting periods for ivGTT in female Holstein Friesian and other breeds during the last 10 yr is provided in Tables 1 and 2, respectively. The studies show diverse fasting periods ranging from 0 to 48 h and variations in glucose dose, which emphasizes the lack of standardization in domestic cattle.

Ruminants have a large number of phylogenetic particularities when compared with nonruminants. For instance, a large percentage of simple sugars and starch derived from the diet are used by ruminal microbes, whereas a small amount is able to pass to the small intestine (reviewed by Lean et al., 2014). It has been suggested that feeding does not cause a significant blood glucose response in adult cattle resulting in lack of necessity of fasting when performing an ivGTT (Kaneko, 2008). However, this hypothesis could lead to misinterpretation because previous studies have found significant differences in plasmatic concentrations of glucose, insulin, and other hormones in fasted lactating and nonlactating Holstein cows and heifers compared with nonfasted animals (Chelikani et al., 2004). Additionally, during the ruminal fermentation process, VFA are synthetized and subsequently used by the liver in a process known as gluconeogenesis (reviewed by Aschenbach et al., 2010). Therefore, alterations in feed intake might modify glucose and insulin concentration as demonstrated by some studies (Danfær, 1994). To our knowledge, no systematic investigation has addressed the effects of fasting period on ivGTT parameters. We hypothesized that ivGTT traits and insulin responses could vary depending on the length of food deprivation. The objective of this study was to determine the influence of 5 different fasting periods on ivGTT parameters in nonpregnant Holstein heifers.

MATERIALS AND METHODS

Animals

Twenty-eight nongestating Holstein Friesian heifers were obtained from a commercial dairy farm located in Northern Germany. At the beginning of the investigation, animals were 12 mo old with an average BW of 441.8 (SD = 3.5 kg). Animals were kept in a freestall with headlocks on a rubber bedding surface.

Study Design

The tests were performed at the farm as a routine procedure. Animals were assigned to 5 groups in a crossover

Table 1. Intravenous glucose tolerance test studies using different fasting periods in female Holstein Friesians in the last 10 yr[1]

Protocol	Fasting duration (h)	Age, weight, or lactation (SD)	Reference
0.25 g of glucose/kg of BW	6	>2 lactations	Abuelo et al. (2016)
0.3 g of glucose/kg of BW (50% dextrose sol)	12	NR	Marett et al. (2015)
150 mg/kg of BW (50% dextrose sol)	2	727 kg (22.3)	Hashemzadeh-Cigari et al. (2015)
1 g of glucose/kg of $BW^{0.75}$	OF	>2 lactations	Hötger et al. (2013)
0.25 g of glucose/kg of BW (50% dextrose sol)	1	NR	Tao et al. (2012)
0.25 g of glucose/kg of BW (50% dextrose sol)	2	1.3 lactation (0.6)	Huzzey et al. (2012)
0.25 g/kg of BW (50% glucose sol)	24–48	NR	Schoenberg et al. (2012)
1 g/kg of $BW^{0.75}$	12	2 lactations	Lohrenz et al. (2010)
50% dextrose sol	0	NR	Wheelock et al. (2010)
0.25 g/kg of BW (50% dextrose sol)	RF	671 (39) to 765 kg (36)	Pires et al. (2008)
0.3 g/kg of BW (50% D-glucose sol)	12	2.5 lactations (1.2)	Roche et al. (2008)
0.3 g of glucose/kg of BW	12	4.6 yr (1.4)	Boston et al. (2008)
0.25 g/kg of BW (50% dextrose sol)	RF	786 kg (36)	Pires et al. (2007)
1.67 mmol of glucose/kg of BW (50% dextrose sol)	RF	675 kg (69)	Bradford and Allen (2007)
0.45 g/kg of $BW^{0.75}$	1	11–14 mo	Sumner et al. (2007)
0.3 g/kg of BW (50% D-glucose sol)	OF	2 yr	Chagas et al. (2006)

[1]sol = solution; NR = not reported; OF = overnight fasting; RF = restricted feeding.