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Evaluation of glucose dose on intravenous glucose tolerance test traits in Holstein-Friesian heifers

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ABSTRACT

Glucose metabolism in dairy and beef cattle has received considerable attention because balanced blood glucose is essential for numerous processes, such as milk production and general health. The glucose tolerance test measures the ability of an organism to regulate blood glucose levels. Glucose half-life time (GHLT) has high heritability and could serve as a potential parameter to breed for metabolic resistance. However, studies focusing on identification of an adequate glucose dose have not yet been conducted in cattle. The objective of this study was to analyze the effect of 5 different glucose doses (0.5, 1, 1.5, 2, and 3 g/kg of body weight$^{0.75}$) on intravenous glucose tolerance test (ivGTT) traits and insulin responses in nongestating heifers. A total of 150 tests were performed in 30 Holstein-Friesian heifers aged 13 to 15 mo. Blood samples were obtained every 7 min after glucose injection until min 63. Glucose traits and insulin parameters included blood serum glucose and insulin concentration at min 0 (basal concentration), min 7 to 21 (peak glucose and insulin concentration), and min 63 (last sampling) relative to glucose administration, glucose and insulin area under the curve (GAUC and IAUC), and GHLT estimated between min 14 and 42. Serum glucose and insulin concentrations were measured according to the hexokinase colorimetric method and radioimmunoassay, respectively. Generalized linear mixed model was used to test for significant differences in ivGTT traits, insulin responses, and glucose elimination rates (k) over time at different glucose doses. Maximum glucose and insulin concentrations at min 63 increased with higher glucose doses ($P < 0.001$). Significantly lower GHLT ($P < 0.001$) were obtained at increasing glucose doses, whereas GAUC and IAUC were significantly higher ($P < 0.001$) at increasing doses. The k values were affected by glucose dose ($P < 0.001$) and by time interval ($P < 0.001$). Glucose dose greatly affected most ivGTT traits, insulin responses, and glucose elimination rates. Therefore, researchers should standardize their methods to achieve repeatable results and use the same time points for GHLT calculation. Higher glucose doses ($\geq$1.5 g/kg of body weight$^{0.75}$) triggered glucose concentrations above the glucose renal threshold during the initial 42 min, whereas the lowest glucose concentration failed to induce a maximum insulin response. Further research is necessary to determine an adequate dose inducing maximum insulin responses with minimum renal glucose losses.
Key words: glucose half-life time, insulin, metabolism, glucose tolerance test

INTRODUCTION

Carbohydrate metabolism has been largely studied in cows because of the essential role that glucose plays in milk synthesis (reviewed by Aschenbach et al., 2010). Insulin is responsible for promoting glucose uptake in numerous tissues expressing insulin-responsive glucose transporters, whereas tissues with high energy demands, such as the mammary gland, use insulin-independent uptake mechanisms (Komatsu et al., 2005). Monitoring insulin responses through an intravenous glucose tolerance test (**ivGTT**) is a routine diagnostic tool used to estimate insulin resistance (De Koster and Opsomer, 2013). Commonly, this procedure uses direct quantification of insulin and metabolites or more complex analyses including mathematical modeling (Boston et al., 2006). The test also provides information to assess the influence of specific diets or medical treatments on glucose metabolism and general health status (Sumner et al., 2007; Grünberg et al., 2011). Furthermore, Pieper et al. (2016) found high heritability for some ivGTT traits, such as glucose half-life time (**GHLT**) in young Holstein bulls, which is related to glucose clearance times from the body. Other parameters derived from the test are influenced by numerous factors besides individual variation (González-Grajales et al., 2017). As suggested by Pieper et al. (2016), GHLT might be used

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in the future as a selection criterion to predict animal health due to the development of numerous conditions in dairy cattle as a result of impaired carbohydrate and lipid metabolism (Drackley et al., 2005). Further studies in this field should aim for a standardization of the test and identification of confounding factors that might alter insulin secretion and glucose utilization rates, such as diet composition (Mears, 1993), fasting times (Kremer, 2008), and glucose dose (Kaneko, 1997).

In young bulls, the glucose tolerance test consists of an intravenously administered glucose solution followed by blood collections every 7 min after injection within 1 to 2 h (Freyer et al., 2004), whereas in lactating cows the examination is commonly extended for 2 h (Hötger et al., 2013). It has been reported that the total glucose amount administered i.v. to ruminants is weight dependent (Kaneko, 1997). Moreover, to our knowledge, the effects of different glucose doses on insulin responses and glucose turnover rates in the same individual are currently unknown. Doses have been calculated on the basis of BW and metabolic BW ($\mathbf{BW^{0.75}}$) and ranged from 0.15 (Hashemzadeh-Cigari et al., 2015) to 0.5 g/kg of BW (Kerestes et al., 2009) and from 0.15 (Hayirli et al., 2001) to 1 g/kg of $BW^{0.75}$ (Hötger et al., 2013). These inconsistencies are illustrated in current studies implementing ivGTT in Holstein females (Table 1). Conversely, in human medicine, glucose is administered orally at a fixed dose of 75 g following guidelines by the World Health Organization (Andersen et al., 2016). Thus, data analyses could be appropriately interpreted because glucose dose is very similar among studies. The lack of standardization of glucose dose in cattle studies (e.g., bulls, heifers, or cows) might lead to misinterpretation due to poor stimulatory insulin responses or prolonged hyperglycemic states. It is imperative to investigate the effect of different glucose doses on glucose tolerance test responses and carefully revise factors that might affect the responses to the glucose dose such as age, plane of nutrition, and physiological state. Therefore, we hypothesized that ivGTT traits and insulin responses vary depending on the glucose dose administered to nongestating heifers. The objective of this study was to determine the influence of 5 different glucose doses on glucose traits, insulin responses, and glucose turnover rates ($\boldsymbol{k}$).

MATERIALS AND METHODS

Heifers

Nongestating Holstein-Friesian heifers (n = 30) were purchased from a commercial farm located in Northern Germany. Clinical examinations and blood collections were conducted in the same farm as a routine procedure. At the beginning of the study, females weighed 422.5 (SD ±33.1 kg) and were 13 to 15 mo old. Animals

Table 1. Intravenous glucose tolerance test studies using different doses in female Holstein-Friesians[1]

Glucose dose	Age, BW (SD)	Lactation (SD), DIM	Reference
Estimated by BW			
0.15 g/kg (50% sol)	Multiparous: 727 kg (22.3)	Dry cows	Hashemzadeh-Cigari et al. (2015)
	Primiparous: 608 kg (15.9)		
0.25 g/kg (50% sol)	Multiparous	≥2 lactations, 21 DIM	Mann et al. (2016)
	Multiparous	>2 lactations	Abuelo et al. (2016)
	Average parity: 1.4 and 1.7	42 DIM	Tao et al. (2012)
	Primiparous and multiparous	Dry cows	Huzzey et al. (2012)
	Multiparous	≥2 lactations, dry cows	Schoenberg et al. (2012)
	655 kg (63)	99.8 d (20.2)	Wheelock et al. (2010)
	671 (39) to 765 kg (36)	Nonlactating, nongestating	Pires et al. (2008)
	786 kg (36)	Nonlactating, nongestating	Pires et al. (2007a)
	722 kg (46)	Nonlactating, nongestating	Pires et al. (2007b)
0.3 g/kg (50% sol)	Primiparous and multiparous	50–150 d	Oliveira et al. (2016)
	Multiparous	100, 250, 460, 560 DIM	Marett et al. (2015)
	Multiparous	2.5 lactations (1.2), 35 DIM	Roche et al. (2008)
	4.6 yr (1.4), 539 kg (72.6)	35 DIM	Boston et al. (2008)
0.3 g/kg (30% sol)	2 yr	1 lactation, 14 DIM	Chagas et al. (2006)
	Primiparous and multiparous 569 (42) to 697 (52) kg	Transition, 14 DIM	Subiyatno et al. (1996)
	Multiparous	2–5 lactations, different DIM	Opsomer et al. (1999)
0.5 g/kg (40% sol)	Multiparous	3–5 lactations, late gestational	Kerestes et al. (2009)
Estimated by metabolic $BW^{0.75}$			
0.15 g/kg (50% sol)	Multiparous 730 kg (61)	28 DIM	Hayirli et al. (2001)
0.45 g/kg	8.1–12.5 mo	Nongestating	Sumner et al. (2007)
1 g/kg	NR	84 DIM	Hötger et al. (2013)
	NR	2 lactations	Lohrenz et al. (2010)

[1]Sol = solution; NR = not reported.