J. Dairy Sci. 101:1–15
https://doi.org/10.3168/jds.2017-12877

Adipose tissue insulin receptor and glucose transporter 4 expression, and blood glucose and insulin responses during glucose tolerance tests in transition Holstein cows with different body condition

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ABSTRACT

Glucose uptake in tissues is mediated by insulin receptor (INSR) and glucose transporter 4 (GLUT4). The aim of this study was to examine the effect of body condition during the dry period on adipose tissue mRNA and protein expression of INSR and GLUT4, and on the dynamics of glucose and insulin following the i.v. glucose tolerance test in Holstein cows 21 d before (d −21) and after (d 21) calving. Cows were grouped as body condition score (BCS) ≤3.0 (thin, T; n = 14), BCS = 3.25 to 3.5 (optimal, O; n = 14), and BCS ≥3.75 (overconditioned, OC; n = 14). Blood was analyzed for glucose, insulin, fatty acids, and β-hydroxybutyrate concentrations. Adipose tissue was analyzed for INSR and GLUT4 mRNA and protein concentrations. During the glucose tolerance test 0.15 g/kg of body weight glucose was infused; blood was collected at −5, 5, 10, 20, 30, 40, 50, and 60 min, and analyzed for glucose and insulin. On d −21 the area under the curve (AUC) of glucose was smallest in group T (1,512 ± 33.9 mg/dL × min) and largest in group OC (1,783 ± 33.9 mg/dL × min), and different between all groups. Basal insulin on d −21 was lowest in group T (13.9 ± 2.32 μU/mL), which was different from group OC (24.9 ± 2.32 μU/mL. On d −21 the smallest AUC 5–60 of insulin in group T (5,308 ± 1,214 μU/mL × min) differed from the largest AUC in group OC (10,867 ± 1,215 μU/mL × min). Time to reach basal concentration of insulin in group OC (113 ± 14.1 min) was longer compared with group T (45 ± 14.1). The *INSR* mRNA abundance on d 21 was higher compared with d −21 in groups T (d −21: 3.3 ± 0.44; d 21: 5.9 ± 0.44) and O (d −21: 3.7 ± 0.45; d 21: 4.7 ± 0.45). The extent of INSR protein expression on d −21 was highest in group T (7.3 ± 0.74 ng/mL), differing from group O (4.6 ± 0.73 ng/mL), which had the lowest expression. The amount of GLUT4 protein on d −21 was lowest in group OC (1.2 ± 0.14 ng/mL), different from group O (1.8 ± 0.14 ng/mL), which had the highest amount, and from group T (1.5 ± 0.14 ng/mL). From d −21 to 21, a decrease occurred in the GLUT4 protein levels in both groups T (d −21: 1.5 ± 0.14 ng/mL; d 21: 0.8 ± 0.14 ng/mL) and O (d −21: 1.8 ± 0.14 ng/mL; d 21: 0.8 ± 0.14 ng/mL). These results demonstrate that in obese cows adipose tissue insulin resistance develops prepartum and is related to reduced GLUT4 protein synthesis. Regarding glucose metabolism, body condition did not affect adipose tissue insulin resistance postpartum.

Key words: insulin receptor, glucose transporter 4, glucose tolerance test, adipose tissue, body condition score

INTRODUCTION

Transition from pregnancy to lactation is associated with important readjustments in metabolism of dairy cows. Due to the onset of milk synthesis, requirements for energy and nutrients, especially for glucose, increase markedly after calving, leading to negative energy balance (**NEB**; Bell, 1995). To compensate for the energy and nutrient deficiency, large quantities of fatty acids are mobilized from adipose tissue (**AT**), which, with concurrent low glucose availability, will support the production of BHB and may lead to the development of ketosis (Oetzel, 2007). Insulin and sensitivity of tissues to insulin play a central role in the adjustment of energy partitioning between tissues and in balancing lipogenesis and lipolysis (Bell and Bauman, 1997). In

Received March 16, 2017.
Accepted August 31, 2017.
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dairy cows, insulin resistance (**IR**) develops during late pregnancy and represents an important homeorhetic adaptation, canalizing metabolism to use energy, mainly stored in AT, to support body functions and to minimize glucose consumption in peripheral tissues, sparing it for milk synthesis under conditions of nutrient and energy deficiency (Bell, 1995; Salin et al., 2012; De Koster and Opsomer, 2013; Zachut et al., 2013).

In general, insulin activates pathways responsible for energy storage within the body [e.g., glucose uptake, lipogenesis, and glycogenesis (Bell and Bauman, 1997)]. In AT, insulin signal transduction starts with binding insulin to its receptor (**INSR**). The consequent intracellular cascade, mainly through the PI3K/AKT/mTOR pathway, promotes the expression, translocation, and fusion with cell membrane of insulin-dependent glucose transporters 4, responsible for insulin-induced glucose uptake from blood in adipose and muscle tissue. Therefore, INSR and GLUT4, incorporated in the membrane, represent the start and end of insulin signaling responsible for facilitated cellular glucose uptake (Lewis et al., 2002).

The plane of nutrition as a factor influencing insulin sensitivity (Holtenius et al., 2003; Schoenberg et al., 2012) and insulin signaling (Mann et al., 2016b) in cows has been extensively investigated. Regarding body fat reserves, both suboptimal body condition and overcondition before parturition are associated with poor adaptation in the subsequent lactation, leading to an increased incidence of metabolic disorders such as ketosis and fatty liver (Drackley, 1999; Bobe et al., 2004; Goff, 2006; Roche et al., 2013a,b), impaired fertility (Samarütel et al., 2008a,b; Roche et al., 2009), and more pronounced IR (Holtenius and Holtenius, 2007; Jaakson et al., 2013). According to De Koster and coworkers (De Koster et al., 2015, 2016a), development of IR in overconditioned cows at the end of the dry period is associated rather with glucose than lipid metabolism. However, despite intense research work, and due to differences in experimental conditions, design, and aims, a clear understanding regarding cause-and-effect relationships between the IR and the physiological and metabolic status of cows, as well as understanding about the molecular mechanisms of insulin signaling are still lacking. Moreover, the number of studies characterizing protein expression of INSR and GLUT4 in AT is limited (Mann et al., 2016b). No integrated studies are available describing AT INSR and GLUT4 expression in relation to BCS both at the mRNA and protein levels.

In this study we hypothesized that the development and extent of IR during the transition period, mediated by the expression and function of INSR and GLUT4, are related to the amounts of body fat reserves in the dry period. Therefore, the aim of this study was to examine the effect of BCS during the dry period on the mRNA and protein expressions of INSR and GLUT4 in s.c. AT, in addition to the dynamics of insulin and glucose following intravenous glucose infusion in Holstein dairy cows 21 d before (d −21) and after (d 21) calving.

MATERIALS AND METHODS

Experimental Design and Animals

The study was carried out on 42 multiparous Holstein cows on the experimental farm of the Estonian University of Life Sciences, which has a herd size of about 120 cows and with average annual milk production of about 9,200 kg per cow. Cows were indoor housed in freestall barns with rubber mats and sawdust bedding and fed TMR ad libitum. Lactating cows were milked twice a day. Three experimental groups were established and proportionally assigned according to the blocked design with each block consisting of 3 cows over 2 consecutive years on the basis of cows' BCS (Edmonson et al., 1989) 28 d before expected calving (d −28) as follows: BCS ≤3.0 (2.25–3.00; thin, **T**; n = 14), BCS = 3.25 to 3.5 (optimal, **O**; n = 14), and BCS ≥3.75 (3.75–4.50; overconditioned, **OC**; n = 14). Parity distribution was different between groups T [2nd to 5th (2.6) parity] and OC [2nd to 6th (3.7) parity; $P = 0.006$]; group O [2nd to 6th (3.2) parity] did not differ from other groups. Fortnightly assessment of the potential experimental cows' BCS began at drying off, on an average 54 (52–59) d before expected calving. Cows with an appropriate BCS, and who had maintained their BCS from drying off until d −28, were assigned to the study blocks. Such cows were removed from the dry cow barn to the tiestall housing to adjust to the experimental conditions. After calving, from the seventh milking, cows were removed to a freestall barn with attached milking parlor.

The European Council Directive regarding the protection of animals, and the Estonian Animal Protection Act, have been complied with in this experiment. The study has been approved by the Committee for Conducting Animal Experiments at the Estonian Ministry of Agriculture.

Feeds and Feeding

Cows were fed grass silage, hay, corn meal, barley meal, heat-treated rapeseed cake, and mineral feeds as TMR twice daily ad libitum around 0530 and 1430 h. Depending on physiological stage and requirements, cows were fed 5 rations differing in chemical composition and nutritive value (Table 1). Rations were calcu-