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Short communication: Relationship between body condition score and plasma adipokines in early-lactating Holstein dairy cows

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ABSTRACT

We hypothesized that plasma adipokine concentrations of early-lactation dairy cows are related to body condition score (BCS) at calving and to markers of metabolic status of the cow. As part of a larger study with 117 multiparous Holstein dairy cows, which had high BCS (BCS >4.0) or normal BCS (3.25–3.5) at calving, 22 cows were randomly selected (n = 11 per group) to be enrolled in this study. Cows were divided into 2 groups based on their BCS at calving: (1) normal BCS with BCS of 3.35 ± 0.13 (mean ± SD) and (2) high BCS cows with BCS of 4.14 ± 0.17. The 22 selected animals did not have a clinically diagnosed health problem after calving. Blood samples were taken right after calving (d 1) and before morning feeding on d 8, 15, and 21 postpartum concurrently with body condition scoring for all cows. Blood samples were analyzed for plasma adiponectin, leptin, tumor necrosis factor-α, and IL-6. The mean BCS remained highest in high-BCS cows during the first 21 d in milk. Leptin concentrations decreased progressively for all cows after calving. However, differences in BCS at calving were not related to leptin concentrations. Adiponectin, IL-6, and tumor necrosis factor-α concentrations were neither influenced by days in milk nor BCS after calving. Leptin and the leptin-to-adiponectin ratio did not show any correlation at any time point during the first 21 d in milk with plasma concentrations of nonesterified fatty acids or β-hydroxybutyrate, which are considered as markers of metabolic status. Only for IL-6 at d 8 did we find a strong correlation with metabolic status indicators. In conclusion, plasma adipokine concentrations during the first 3 wk postpartum were not related to BCS in lactating Holstein cows that were clinically healthy at calving.

Key words: dairy cow, leptin, adiponectin, tumor necrosis factor-α, interleukin-6

Short Communication

Adipose tissue is an important endocrine organ that secretes several bioactive molecules termed adipokines, which are involved in the regulation of whole body metabolism and immune responses (Fantuzzi, 2005; Trayhurn et al., 2006). Adiponectin and leptin are among the most important adipokines in the control of energy homeostasis (Havel, 2002). In humans, plasma leptin concentrations are positively correlated with degree of adiposity (Considine et al., 1996) and insulin resistance (Matsubara et al., 2000). In general, low concentrations of adiponectin are associated with an obese phenotype and with the release of inflammatory proteins (Weyer et al., 2001; Yang et al., 2001). Concentrations of these 2 adipokines in plasma have been associated with the occurrence of certain metabolic diseases, and it has been proposed that they can be used as chemical markers for diagnosis of obesity and obesity-related disorders in humans (Considine et al., 1996; Shimomura et al., 1999). Moreover, the ratio of leptin to adiponectin has been suggested as a potential index for obesity in some mammalian species, such as cynomolgus monkeys (Chen et al., 2003), and as a potential index for coexistence of obesity and type 2 diabetes mellitus in human patients (Satoh et al., 2004). Concentrations of other adipokines in plasma are also increased in the obese state. Tumor necrosis factor-α (**TNF-α**) and IL-6 are 2 important proinflammatory adipokines that promote insulin resistance, and they have been linked with obesity-related diseases such as diabetes type 2 (Goyal et al., 2012). In addition, it is known that adiponectin is correlated negatively with TNF-α and IL-6 (Lira et al., 2011). Concentrations of IL-6 in plasma are positively

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associated with the severity of inflammatory response in early-lactating dairy cows (Trevisi et al., 2012), and IL-6 has been suggested as a predictive marker for diagnosis of animals that may fail to have a successful transition period (Trevisi et al., 2015).

It has been documented that cows with a high BCS at calving have lowered postpartal daily DMI and experience a more severe negative energy balance (**NEB**) with more extensive mobilization of body fat postpartum (Roche et al., 2009). Most studies claim that NEB is the most influential factor in predisposing overconditioned cows to different postpartal metabolic diseases (Hayirli et al., 2002; Roche et al., 2009). However, knowledge is lacking regarding the role played by the adipose-derived adipokines in regulation of metabolic status and inflammation and in the development of transition disorders in early-lactating dairy cows. We hypothesized that concentrations of important adipose-derived adipokines in plasma in early-lactating dairy cows are related to BCS of the cow at calving and that concentrations of these adipokines can be used as markers for metabolic status of the early-lactating dairy cow. The objectives of this study were to determine (1) whether BCS and BCS changes across the postpartal period are related to concentrations of adipose-derived adipokines in plasma in early lactation and (2) whether changes in concentrations of adipokines in plasma in early lactation are related to changes in commonly used metabolic markers.

The Committee for Animal Care and Use, University of Zanjan, Iran, approved the experimental and management protocols of this study. Body condition score was scored immediately after parturition (1 DIM) based on the 1 to 5 scale described by Ferguson et al. (1994). Among a larger group of 117 cows, 11 clinically healthy cows with a BCS between 3.25 and 3.5 at calving were assigned to a normal-BCS (**NBCS**) group, and 11 clinically healthy cows with a BCS $\geq$4 were assigned to a high-BCS (**HBCS**) group. Before parturition, all cows had been fed the same diet, which was formulated to meet energy and nutritional requirements according to the NRC (2001). All animals were fed the same diet postpartum, which was formulated to meet requirements for lactating dairy cows according to the NRC (2001).

Throughout the experimental period, the cows were housed in open barns with straw bedding. Both pre- and postpartum diets were offered ad libitum as a TMR twice daily (at 0600 and 1430 h). Fresh water was available at all times for all animals. Early-lactating cows were milked 3 times daily at 0500, 1230, and 2000 h. Milk yield was recorded once weekly at d 8, 15, and 21 postpartum. Concurrently, milk samples from each of the 3 daily milkings were taken, pooled, and stored at −21°C until analysis for fat and protein concentrations by using a Milko-Scan 33 (Foss Electric, Hillerød, Denmark). Energy-corrected milk (4% fat, 3.3% protein) was calculated using the following equation: ECM = (milk production × 0.383 × fat % + milk production × 0.242 × protein % + 0.7832 × milk production)/3.1138. Blood samples were collected by venipuncture from the coccygeal vein right after birth (d 1) and on d 8, 15, and 21 postpartum before the morning feeding. Blood was collected into heparinized vacutainer tubes, and plasma was separated immediately after by centrifugation at 1,300 × *g* for 15 min at 4°C. Aliquots of plasma were stored at −80°C for later analyses. Plasma leptin was analyzed with an enzyme immunoassay that has been validated for use in several species, including bovine (Sauerwein et al., 2004). The mean intra-assay coefficient of variation was 3%, and the inter-assay coefficient of variation was 3.4%. Plasma adiponectin was analyzed by a competitive ELISA, as described by Mielenz et al. (2013). The intra- and inter-assay coefficients of variation were 5.6 and 8.2%, respectively. Concentrations of IL-6 and TNF-α in plasma were determined by commercial high sensitive ELISA kits for bovine IL-6 (kit SEA079Bo; USCN Life Science, Wuhan, China) and bovine TNF-α (kit Q06599; Thermo Fisher Scientific Inc., Waltham, MA), respectively. Nonesterified fatty acid (**NEFA**; Wako Chemicals, Neuss, Germany; kit 436–91995) and BHB (kit RB 1008; Labor und Technik, Berlin, Germany) concentrations were measured in plasma by using an ABX Pentra 400 instrument (Horiba Medical, Kyoto, Japan).

All data were analyzed by using SPSS 21.0 (SPSS Inc., Chicago, IL). Analysis of variance for repeated measures was used to test the effect of BCS through time for all blood parameters. In this analysis, time was considered repeated with 4 levels [1 (day of calving), 8, 15, and 21 DIM]. In the analyses of blood parameters, within-animal factors and group as the between-animal factor were included in the models to test for differences between the 2 groups of animals with respect to changes of parameters over the first 21 d postpartum. Tukey test was used for the determination of differences between BCS groups. Sphericity was checked for violation with Greenhouse–Geisser corrected degrees of freedom, if this was needed (Huck, 2000). For all analyses, homogeneity of variances was tested using the Levene's test. Differences at each time point between HBCS and NBCS groups were compared by Student's *t*-test. Pearson correlation coefficients were calculated to describe relationships between the monitored variables. Moreover, simple linear regression analyses were performed to test the independent relations between variables that were found to be significantly correlated. Confidence interval was set at 95%. Significance and tendency dif-