J. Dairy Sci. 96:5656–5669
http://dx.doi.org/10.3168/jds.2012-6142

Periparturient dairy cows do not exhibit hepatic insulin resistance, yet adipose-specific insulin resistance occurs in cows prone to high weight loss

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ABSTRACT

The periparturient period in dairy cows is associated with alterations in insulin action in peripheral tissues; however, the molecular mechanism underlying this process is not completely understood. The objective was to examine the response to a glucose tolerance test (GTT) and to analyze insulin signaling in liver and adipose tissues in pre- and postpartum dairy cows. Liver and adipose tissue biopsies were taken before and after GTT, at 17 d prepartum and again at 3 to 5 d postpartum from 8 high-yielding Israeli Holstein dairy cows. Glucose clearance rate after GTT was similar pre- and postpartum. Basal insulin concentrations and the insulin response to GTT were approximately 4-fold higher prepartum than postpartum. In accordance, phosphorylation of the hepatic insulin receptor after GTT was higher prepartum than postpartum. Across periods, a positive correlation was observed between the basal and peak plasma insulin and phosphorylated insulin receptor after GTT in the liver. Hepatic phosphorylation of protein kinase B after GTT was elevated pre- and postpartum. Conversely, in adipose tissue, phosphorylation of protein kinase B after GTT pre- and postpartum was increased only in 4 out of 8 cows that lost less body weight postpartum. Our results demonstrate that hepatic insulin signaling is regulated by plasma insulin concentrations as part of the homeorhetic adjustments toward calving, and do not support a model of hepatic insulin resistance in periparturient cows. Nevertheless, we suggest that specific insulin resistance in adipose tissue occurs pre- and postpartum only in cows prone to high weight loss. The different responses among these cows imply that genetic background may affect insulin responsiveness in adipose tissue pre- and postpartum.

Key words: peripartum insulin resistance, insulin receptor, protein kinase B (Akt)

INTRODUCTION

The modern high-yielding dairy cow faces a great metabolic challenge during the peripartum period: a shift from a nonlactating, late-gestational, and lipogenic period to a state of tremendous energy demand for milk production. This results in massive lipolysis of adipose and catabolism of muscle tissues, as the cow is not able to consume sufficient nutrients to meet the energy requirements of the mammary gland (Bell and Bauman, 1997). In fact, the demand for glucose increases by 4 fold from late pregnancy to early lactation in dairy cows (Bell, 1995). Because the main source of glucose in ruminants is the conversion of propionate to glucose through hepatic gluconeogenesis, the liver adjusts to the high rate of gluconeogenesis postpartum by increasing the expression of key gluconeogenic enzymes such as pyruvate carboxylase (**PC**) and phosphoenolpyruvate carboxykinase (**PEPCK**; Graber et al., 2010).

Insulin plays a pivotal role in the physiological adjustments occurring around parturition in dairy cows (i.e., shifting from lipogenesis to lipolysis) while maintaining high glucose availability for the mammary gland. Bell and Bauman (1997) suggested that the marked reduction in whole-body glucose oxidation in newly parturient ruminants (Bauman and Elliot, 1983) implies reduced glucose use by peripheral tissues mediated by very low plasma insulin levels, and possibly tissue refractoriness to insulin. Insulin resistance is defined as a state in which the sensitivity of target cells to respond to ordinary levels of insulin is reduced (Boura-Halfon and Zick, 2009); therefore, ordinary levels of insulin fail to trigger its metabolic actions. It was concluded that the multiple adaptations from late pregnancy to lactation in dairy cows were at least partly mediated by development of insulin resistance in maternal peripheral tissues (Bell and Bauman, 1997). Indeed, in rats and in humans, it is well accepted that late pregnancy is characterized by a state of insulin resistance, which diminishes after parturition (Ryan et al., 1985; Sevillano et al., 2007).

Insulin action is initiated upon binding of insulin to its receptor, which activates the intrinsic tyrosine

Received September 10, 2012.
Accepted May 4, 2013.
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kinase activity of the insulin receptor (**IR**). Phosphorylation of Tyr residues of target proteins by the activated IR kinase triggers the propagation of 3 major signaling pathways: the phosphatidylinositol 3-kinase (PI3K)/ protein kinase B (**Akt**); rat sarcoma/rapidly accelerated fibrosarcoma/extracellular signal-regulated kinase (Ras/Raf/ERK), and the Cbl/Cbl-associated protein (CAP) pathways, all promoting the metabolic and growth-promoting functions of insulin (Saltiel and Kahn, 2001).

Because the decline in insulin concentrations during the periparturient period as a homeorhetic regulation is evident, we hypothesized that this reduces insulin inhibition in lipolysis and gluconeogenesis. Consequently, an increase in lipids breakdown and glucose production occurs, while sustaining normal response to insulin in the target tissues. Therefore, the aim of the present work was to determine whether insulin signaling supports a mechanism of insulin resistance in the liver and adipose tissues during the periparturient period. To this end, we analyzed the effects of a glucose load on key elements along the insulin signaling pathway in pre- and postpartum dairy cows in biopsies of liver and adipose tissue.

MATERIALS AND METHODS

The experimental protocol of the study was approved by the Volcani Center Animal Care Committee and was conducted at the Volcani Center experimental farm in Bet Dagan, Israel. Eight high-yielding, 261 ± 5 d pregnant, nonlactating Israeli Holstein dairy cows, which averaged 740 ± 73 kg of live BW and average lactation number 4.6, participated in this study. Cows were group housed in covered loose pens with adjacent outside yards and fed ad libitum once per day at 1100 h with standard Israeli diets. The pre- and postpartum diets contained 1.46 and 1.77 Mcal of NE_L/kg of DM and 13.2 and 16.6% CP, respectively (Table 1). After calving, cows were milked 3 times daily and milk production was recorded electronically (SAE Afikim, Kibbutz Afikim, Israel). One cow was excluded from analysis of milk production due to subclinical mastitis that led to culling at 60 d postpartum. The cows were weighed prepartum once per week, and postpartum were automatically weighed 3 times daily after each milking with a walking electronic scale (SAE Afikim). Body condition score was determined by a single technician on a scale of 1 to 5 (1 = lean, 5 = obese) at 261 d of pregnancy, and 3 to 5, 14, 28, and 117 ± 3 d postpartum.

Blood samples were collected 3 times weekly (Sunday, Tuesday, and Thursday) from 21 d before the expected calving until 21 d postpartum from the jugular vein into vacuum tubes containing lithium heparin (Becton Dickinson Systems, Cowley, UK). The blood samples were collected after morning milking at 0800 h and plasma was separated after centrifugation at 4,000 × *g* for 15 min and stored at −32C pending analysis.

Glucose Tolerance Test

At late pregnancy (261 ± 5 d of pregnancy), 3 to 5 d postpartum, and at mid lactation (117 ± 3 d postpartum), cows received an intravenous glucose challenge after the morning milking. On the day before the glucose challenge, a catheter (14 G × 13 cm; Mila International, Erlanger, KY) was inserted into the jugular vein of each cow under local anesthesia to facilitate frequent blood collection. Food was withheld for 12 h before the glucose challenge and free access to water was maintained. Glucose was warmed to body temperature (38C) and administered intravenously as a 50% solution (Teva Medical Ltd., Ashdod, Israel) at 300 mg of D-glucose/kg of BW and at a constant rate by manually compressing the glucose pack. The infusion of glucose was completed within 3 min. Blood samples were collected at 25 and 20 min before the start of the infusion, just before the infusion (time $t = 0$), every 5 min from $t = 5$ to $t = 45$ min and then at 60 and 75 min after the infusion. Blood samples for insulin and NEFA analysis were collected into blood tubes containing lithium heparin (Becton Dickinson Systems). Additional blood samples for glucose analysis were collected into tubes containing lithium chloride and L-iodoacetate (BD Vacutainer; Belliver Industrial Estate, Plymouth, UK), and were immediately placed in ice. Blood samples were centrifuged within 30 min of collection at 4,000 × *g* for 20 min and stored at −32°C awaiting analysis. Two cows were excluded from analysis of glucose tolerance test (**GTT**) response prepartum; one due to technical failure of glucose infusion, and the other due to postponed calving. To obtain reference values of plasma glucose and insulin, GTT were performed in 4 of the cows at mid lactation (on average, 117 ± 3 d postpartum) that were available at the farm at that time.

Chemical Analysis

Concentrations of plasma insulin were determined by RIA (Diagnostic Products Corp., Los Angeles, CA). The intra- and interassay coefficients of variation for insulin assay were 7.2 and 5.1%, respectively. Plasma glucose was determined by a glucose reagent kit (Glucose UV 10 × 50 mL; Raichem, San Diego, CA). Plasma NEFA concentrations were determined by a kit (Wako NEFA C test kit; Wako Chemicals GmbH, Neuss, Germany).