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Supplementation with conjugated linoleic acids extends the adiponectin deficit during early lactation in dairy cows

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ABSTRACT

Decreasing insulin sensitivity (IS) in peripheral tissues allows for partitioning nutrients towards the mammary gland. In dairy cows, extensive lipid mobilization and continued insulin resistance (IR) are typical for early lactation. Adiponectin, an adipokine, promotes IS. Supplementation with conjugated linoleic acids (CLA) in rodents and humans reduces fat mass whereby IR and hyperinsulinemia may occur. In dairy cows, CLA reduce milk fat, whereas body fat, serum free fatty acids and leptin are not affected. We aimed to investigate the effects of CLA supplementation on serum and adipose tissue (AT) adiponectin concentrations in dairy cows during the lactation driven and parity modulated changes of metabolism. High yielding cows ($n = 33$) were allocated on day 1 post partum to either 100 g/day of a CLA mixture or a control fat supplement (CON) until day 182 post partum. Blood and subcutaneous (sc) AT (AT) biopsy samples were collected until day 252 post partum to measure adiponectin. Serum adiponectin decreased from day 21 pre partum reaching a nadir at calving and thereafter increased gradually. The distribution of adiponectin molecular weight forms was neither affected by time, parity nor treatment. Cows receiving CLA had decreased serum adiponectin concentrations whereby primiparous cows responded about 4 weeks earlier than multiparous cows. The time course of adiponectin concentrations in sc AT (corrected for residual blood) was similar to serum concentrations, without differences between CLA and CON. CLA supplementation attenuated the post partum increase of circulating adiponectin thus acting towards prolongation of peripartal IR and drain of nutrients towards the mammary gland.

1. Introduction

The progression from pregnancy to lactation is characterized by comprehensive metabolic and endocrine changes. Fetal growth, lactogenesis and galactopoiesis require the targeted partitioning of nutrients towards the placenta and the mammary gland, a process that is accomplished by decreasing insulin sensitivity (IS) in peripheral tissues thus attenuating their uptake of glucose, amino acids and fatty acids and facilitating lipolysis in adipose tissue (AT) (Block et al., 2001). Considering the amount of nutrient output via milk, dairy cows present a biological extreme: in contrast to rodents and primates, in which insulin resistance (IR) develops during pregnancy and fades after parturition, dairy cows may maintain IR for several weeks and may excessively mobilize body fat (Tamminga et al., 1997). Adipose tissue is a metabolically active tissue, communicating with other peripheral tissues and brain through secretion of bioactive molecules collectively termed as 'adipokines'. The circulating leptin concentrations reportedly change during pregnancy and around parturition (Block et al., 2001; Sattar et al., 1998) and are affected by parity in dairy cows (Wathes et al., 2007). Parity is considered as an important factor affecting metabolic and hormonal changes, since primiparous cows have not reached their adult body size and continue to grow during pregnancy and lactation thus showing significant differences in metabolism and lipolytic response when compared to later lactations (Theilgaard et al., 2002; Wathes et al., 2007).

Abbreviations: ALR, adiponectin leptin ratio; AT, adipose tissue; BCS, body condition score; CLA, conjugated linoleic acids; CON, control; DM, dry matter; HMW, high molecular weight; IS, insulin sensitivity; IR, insulin resistance; MMW, medium molecular weight; MP, multiparous; NEFA, nonesterified fatty acids; NE_L, net energy for lactation; PMR, partial mixed ration; PP, primiparous; RQUICKI, revised quantitative insulin sensitivity check index; sc, subcutaneous.

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Adiponectin is one of the most abundant adipokines in circulation (Hotta et al., 2001). Unlike other adipokines which increase with excess body fat mass, adiponectin is decreased in obese subjects (Kadowaki and Yamauchi, 2005). Adiponectin inhibits lipolysis in AT and decreases IR by stimulating fatty acid oxidation and reducing the triglyceride content in muscle and liver (Yamauchi et al., 2001). The low adiponectin expression in AT after onset of lactation, might contribute to the decrease in IS, which improves glucose supply for milk synthesis (Komatsu et al., 2007). Adiponectin is synthesized as a 30 kDa monomer and is subsequently assembled to various oligomers detectable as low molecular weight trimers, medium molecular weight (MMW) hexamers and high molecular weight (HMW) oligomers (Waki et al., 2003). The HMW isoform is the biologically active form of adiponectin since it is associated with activation of AMP activated protein kinase in muscle (Waki et al., 2003) and improvement in IS (Pajvani et al., 2003). Recently, the adiponectin: leptin ratio (ALR) was proposed as a more effective and reliable marker of IS (Inoue et al., 2005) and metabolic syndrome (Mirza et al., 2001) than adiponectin or leptin alone.

The term conjugated linoleic acids (CLA) refers to a mixture of positional and geometric isomers of octadecadienoic acids, a naturally occurring group of dienoic derivatives of linoleic acid. Supplements containing the two main isomers *cis*-9, *trans*-11 and *trans*-10, *cis*-12 with a 50:50 ratio have been demonstrated to promote fat loss in rodents and obese or overweight humans and also to prevent body fat accumulation in mice (Park et al., 2007). Adverse side effects of using CLA mainly concern the induction of fatty liver and IR (Poirier et al., 2005; Riserus et al., 2002). In dairy cows, CLA supplementation mainly concerns the mammary gland by reducing milk fat synthesis, whereas body fat and lactation induced lipolysis remain unaffected (Pappritz et al., 2011). In the present study, we hypothesized that for the homeorhetic adaptations to lactation, tissue and circulating adiponectin concentrations will be in support of peripartal IR and that the response to CLA will differ depending on stage of lactation and parity.

The objectives of the present study were (I) to characterize circulating adiponectin concentrations from late pregnancy to early lactation and then throughout lactation in dairy cows, (II) to evaluate potential effects of long term CLA supplementation and parity on circulating adiponectin and characterization of its molecular weight forms, (III) to compare the adiponectin serum concentration with ALR, (IV) to identify changes of the adiponectin concentrations in subcutaneous (sc) AT around parturition and early lactation, and (V) to characterize the relationship of insulin, IGF-1, nonesterified fatty acids (NEFA) concentrations in blood, body condition and systemic IS with adiponectin, leptin and ALR.

2. Materials and methods

2.1. Animals and treatments

This study was conducted at the experimental station of the Friedrich Loeffler Institute, Federal Research Institute for Animal Health, Braunschweig, Germany. All animal experiments were approved by the Lower Saxony state office for consumer protection and food safety (LAVES, file No. 33.11.42502-04-071/07, Oldenburg, Germany). The experimental design has been described in detail elsewhere (Pappritz et al., 2011). Briefly, pregnant German Holstein Friesian cows either multiparous (MP, 2–4 preceding lactations, $n = 22$) or primiparous (PP, first pregnancy, $n = 11$) were studied from day (d) 21 pre partum until d 252 post partum. In period 1 (d 21 pre partum until the day of calving), all animals were housed in group pens according to their feeding group and had free access to water. The diet was a partial mixed ration (PMR) consisting of 63% silage and 37% concentrate (6.8 MJ NE_L/kg DM) on dry matter (DM) basis. The diets were formulated according to the recommendation of the Society of Nutrition Physiology (GfE, 2001). On d 1 post partum, the animals were randomly allocated to either the control group (CON; 11 MP cows and 6 PP cows) or the treatment group (CLA; 11 MP cows and 5 PP cows). In period 2 (from d 1 until d 182 post partum), the CLA animals received 100 g/d of a lipid encapsulated rumen-protected commercial CLA preparation (Lutrell® Pure, BASF SE, Ludwigshafen, Germany). The CLA supplement contained 12% each of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA isomers of total fatty acid methyl esters. The animals consumed 7.6 g/d each of the *trans*-10, *cis*-12 and the *cis*-9, *trans*-11 CLA isomer (calculated, based on the analyzed proportions in the concentrate). The CON group received 100 g/d of a rumen-protected fat preparation (Silafat®, BASF SE) in which the CLA were substituted by stearic acid. During period 2, each cow of both groups received 4 kg/d additional concentrate (8.8 MJ NE_L/kg DM) containing the respective fat supplement. The detailed composition of the diet and the fatty acid profile of the fat supplements are provided elsewhere (Pappritz et al., 2011). To identify potential post supplementation effects, the animals were observed for further 12 weeks after the end of supplementation period (defined as period 3). The body condition score (BCS) of each animal was recorded at the blood sampling times according to a 5-point scale (1 = lean, 5 = fat) as previously described (Edmonson et al., 1989).

2.2. Blood sample collection, adipose tissue biopsies and preparation of tissue extracts

Blood samples from all animals were collected from the jugular vein on d −21, −14, −7, 1, 7, 14, 21, 35, 49, 70, 105, 140, 182, 189, 196, 210, 224, 238 and 252 relative to parturition. The plasma (heparin and EDTA) and serum samples were obtained following standard procedures and were stored at −80 °C. Subcutaneous fat samples were obtained by biopsy as described previously (Saremi et al., 2012) from the tail head region at d −21, 1, 21, and 105 relative to parturition. Tissue samples were immediately snap frozen in liquid nitrogen, and then stored at −80 °C. For determining the adiponectin concentrations in AT, the samples were homogenized in 2 volumes of homogenization buffer, i.e. 10 mM HEPES pH 7.4 with complete® protease inhibitor cocktail (Roche, Mannheim, Germany; 1 tablet/10 mL buffer) using a Precellys® 24 homogenizer in 2 mL tubes containing 1.4 mm zirconium oxide beads (Peqlab Biotechnologies GmbH, Erlangen, Germany). The homogenates obtained were centrifuged twice (14,000*g*, 10 min, 4 °C) to separate the fat layer. The infranatants beneath were collected (without tissue debris) and stored at −80 °C.

2.3. Adiponectin measurements in serum and adipose tissue

Serum samples and AT preparations were analyzed for adiponectin in duplicate using an in-house developed ELISA as described in detail earlier (Mielenz et al., 2013). Assay accuracy was confirmed by linearity and parallelism of diluted serum samples. The measuring range (consistent with the linear range) of the assay was 0.07–1.0 ng/mL and the limit of detection was 0.03 ng/mL. The intra- and interassay coefficients of variation (CVs) were 7 and 9%, respectively.

Correction for residual blood in the tissue extracts was done by comparing the transferrin (Tr) content of the tissue preparation to the Tr content of serum. Transferrin concentrations in each tissue preparation and the corresponding serum sample were determined by an ELISA specific for bovine Tr according to the manufacturer's directions (Bethyl Laboratories Inc., Montgomery, TX) with minor modifications. Briefly, microtiter plates (EIA plate 9018; Corning