Comparative Immunology, Microbiology and Infectious Diseases 37 (2014) 49–58

Contents lists available at ScienceDirect

Comparative Immunology, Microbiology and Infectious Diseases

journal homepage: www.elsevier.com/locate/cimid

Adiponectin links adipose tissue function and monocyte inflammatory responses during bovine metabolic stress

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ARTICLE INFO

Article history:
Received 3 April 2013
Received in revised form
10 September 2013
Accepted 30 October 2013

Keywords:
Parturition
Adiponectin
NEFA
Bovine monocyte

ABSTRACT

The periparturient period of dairy cows is characterized by intense lipid mobilization from adipose tissue leading to increased plasma concentrations of nonesterified fatty acids (NEFA). High NEFA are a predisposing factor for inflammatory based diseases. A major component of these diseases is uncontrolled macrophage/monocyte inflammatory responses. Changes in the endocrine activity of adipose tissue during the periparturient period could impact macrophage function by modifying the secretion of adipokines including adiponectin. Currently, the effects of adiponectin on monocyte activation in dairy cattle are unknown. In humans and rodents, this adipokine regulates monocyte phenotype and alterations in its plasma levels are linked with the development of inflammatory diseases. The objectives of this study were to establish associations between plasma adiponectin expression dynamics and different markers of lipid mobilization during the periparturient period of dairy cows and to characterize the effects of adiponectin on the inflammatory response of bovine monocytes. Plasma adiponectin, NEFA, BHB, albumin, and subcutaneous and retroperitoneal fat depots depth were measured during the periparturient period of dairy cows. In vitro, bovine monocytes were cultured with adiponectin to assess changes in pro-inflammatory responses following LPS stimulation. Results from this study demonstrate that alterations in plasma adiponectin levels in periparturient cattle are inversely correlated with the concentrations of plasma NEFA, an important marker of lipid mobilization. Furthermore, adiponectin exposure significantly decreased monocyte expression of TNFα after LPS stimulation thus markedly reducing their inflammatory response. Reduced plasma adiponectin during the periparturient period could predispose dairy cows to the development of uncontrolled monocyte inflammatory responses.

1. Introduction

Periparturient dairy cattle are more susceptible to inflammatory diseases including mastitis, metritis and laminitis [1]. The high economic impact of these diseases is reflected in increased mortality and culling rates around parturition and during early lactation. In fact, 57% of cows that died while in the herd do so during the first week after calving compared to ~20% after 120 days in lactation [2]. A major risk factor associated with increased morbidity and mortality rates in this group of cows is the enhanced lipid mobilization from adipose tissue depots leading to

Abbreviations: AST, aspartate aminotransferase; BHB, β-hydroxybutytyrate; BUN, blood urea nitrogen; NEFA, nonesterified fatty acids; TNFα, tumor necrosis factor alpha.

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http://dx.doi.org/10.1016/j.cimid.2013.10.007

increased concentrations of plasma NEFA and BHB [3]. High plasma NEFA are directly linked with the development of oxidative stress and uncontrolled inflammatory responses [4]. Activated cells of the monocyte/macrophage lineage are a major cell type associated with inflammation-induced tissue damage, especially through increased TNFα production [5]. Research in humans and rodents suggests that changes in the endocrine activity of adipose tissue can impact macrophage function by modifying the secretion of adipose derived peptides (adipokines) including adiponectin [6,7]. A major gap in our understanding of inflammatory responses in periparturient cows is how increased adipose tissue activity around parturition could lead to uncontrolled monocyte/macrophage inflammatory responses.

Adiponectin is secreted primarily by adipose tissue, however it is also expressed by cardiomyocytes and skeletal muscle [8]. In humans and rodents, adiponectin is found in plasma as 3 major oligomeric forms and a fraction: low-molecular weight (LMW), middle molecular weight (MMW), high molecular weight (HMW), and globular adiponectin [9]. This adipokine possesses important metabolic functions including enhancement of insulin sensitivity, appetite stimulation and the promotion of liver and muscle fatty acid β-oxidation [9]. In females, adiponectin expression is especially influenced by gestation, lactation, and disease. In women, adiponectin expression is reduced by 20% in the last trimester of gestation [10,11]. Hypoadiponectinemia is linked with the pathophysiology of inflammatory diseases including obesity, metabolic syndrome, and atherosclerosis [12]. This could be related to the potent anti-inflammatory effects of the adipokine in macrophages and other immune cells. Recent studies in human monocytes demonstrated that adiponectin reduces their TNFα gene and protein expression [13].

Recently, 3 different groups using diverse approaches reported the dynamics of plasma adiponectin in dairy cows. Initially, Ohtani et al., demonstrated by RIA that plasma adiponectin concentration reach its nadir during the first 2 days after parturition and peaks by 40–70 days into lactation [14]. These findings were further confirmed independently by Mielenz et al., and Giesy et al., using western blotting and ELISA techniques with antibodies raised against bovine adiponectin [15,16]. When analyzing adiponectin oligomer distribution in dairy cows, it was found that in contrast to rodents and humans, circulating adiponectin is composed mainly of high molecular complexes and this distribution is not affected by lactation stage [16].

Despite the importance of adiponectin in metabolic homeostasis and its influence on macrophage inflammatory phenotype in humans and rodents, the effects of this adipokine on the expression of pro-inflammatory cytokines in bovine macrophages are unknown. In the present study we confirmed previous observations regarding changes in plasma total adiponectin during the periparturient period of mature dairy cows. We also established that plasma adiponectin during the periparturient period of mature dairy cows is inversely correlated with plasma concentrations of nonesterified fatty acids (NEFA), an important marker of lipid mobilization from adipose tissue. Furthermore, we examined how adiponectin directly affected primary bovine macrophage activation and confirmed that adiponectin reduces TNFα production in these cells.

2. Methods

2.1. Animals and diets

All animal procedures were approved by the Michigan State University Animal Care and Use committee. Twelve healthy, mature, pregnant Holstein cows in their second or third lactation were selected from a commercial Michigan dairy herd. At the moment of selection, these cows were less than 210 days of gestation, had the last DHI test with SCC <250,000 cells/mL, and a had a body condition score of 3.5–3.75. Animals were monitored daily by experienced farm personnel throughout the periparturient period and early lactation and displayed no signs of clinical diseases. Animals were housed in freestalls and fed a transition diet from 4 weeks before parturition up to calving and a lactation diet (Supplemental Table 1). Subcutaneous (SC) and retroperitoneal (RPT) adipose depot depths were measured by ultrasound examination at the time of blood collection using a Tringa Linear Ultrasound equipped with a linear 5.0 MHz probe (Pie Medical, Maastricht, The Netherlands). The subcutaneous measurement was obtained between the caudal one-quarter and one-fifth connection line going from the dorsal part of the tuber ischia (pins) to the tuber coxae (hooks) as previously described [17]. The retroperitoneal measurement was obtained by positioning the probe on the right inguinal region, dorsolateral to the anterior half of the mammary gland following a perpendicular line from the tuber coxae (Fig. 3). Coefficients of measurement variation (SC = 5.4% and RPT = 9.7%) were calculated based on 3 consecutive measurements at each site.

2.2. Blood sampling and analysis

Blood samples were collected at the following stages during the lactation cycle: dry (27.8d ± 8.2 before calving), closeup (4.6d ± 2.9 before calving), calving (6.1d ± 2.7) and lactation (28.8d ± 5.6). Whole blood was collected via coccygeal venipuncture using Vacuette EDTA blood collection tubes (Greiner Bio-one, Frichenhausen, Germany). A subsample of blood (5 mL) was collected for RNA harvesting. Samples were then centrifuged for 30 min at 1000 × *g* 15 °C and the plasma fraction was collected, aliquoted and stored in a −80 °C freezer for further analysis.

A subsample of plasma (10 mL) was used to perform metabolic profiling at the Diagnostic Center for Population and Animal Health (Michigan State University, East Lansing, MI). All plasma components were analyzed using an autoanalyzer (ADVIA 1650 Chemistry System, Siemens Medical Solution, Tarrytown, NY). β-Hydroxybutytyrate (β-HBA) was measured using the Catachem kit (C442-OA), (Oxford, CT). In this assay, β-HBA dehydrogenase catalyzes the conversion of β-HBA to acetoacetate. During this reaction NAD is reduced to NADH. The change in NADH causes an increase in absorbance that is