J. Dairy Sci. 101:1–14
https://doi.org/10.3168/jds.2017-13777

Influence of adipocyte size and adipose depot on the number of adipose tissue macrophages and the expression of adipokines in dairy cows at the end of pregnancy

E. Depreester,*[1] J. De Koster,†[1,2] M. Van Poucke,‡ M. Hostens,* W. Van den Broeck,§ L. Peelman,‡ G. A. Contreras,† and G. Opsomer*

*Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium
†Department of Large Animal Clinical Sciences, Michigan State University, 736 Wilson Road, East Lansing 48824
‡Department of Nutrition, Genetics and Ethology, Faculty of Veterinary Medicine, Ghent University, Heidestraat 19, 9820 Merelbeke, Belgium
§Department of Morphology, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

ABSTRACT

The aim of this study was to determine the number of adipose tissue macrophages (ATM) and the mRNA expression of adipokines [adiponectin (*ADIPOQ*), leptin (*LEP*), interleukin 6 (*IL6*), tumor necrosis factor (*TNF*), and interleukin 10 (*IL10*)] in different adipose depots from cows with a variable body condition score (BCS) at the end of the dry period. We hypothesized that the number of ATM and the expression of these adipokines depend on adipocyte size and the anatomical location of the adipose depot. Subcutaneous, omental, mesenteric, perirenal, and intrapelvic adipose tissue samples were taken immediately after euthanasia of 10 Holstein Friesian dairy cows (upcoming parity 2 to 5, age 3.9 ± 1.4 yr; mean ± standard deviation) at the end of pregnancy (actual days of pregnancy at the moment of euthanasia: 269 ± 5 d). During the dry period, all animals received similar diets to meet but not exceed requirements. Five animals were considered to have a normal BCS (2.5–3.5) and 5 animals were considered to be over-conditioned (BCS = 3.75–5). Body weight of the animals at the moment of euthanasia was 717 ± 77 kg. Expression of the different genes was determined by reverse transcription quantitative real-time PCR. Adipocyte size was determined by measuring the area of 100 adipocytes on histological sections. Average adipocyte area was 10,475 ± 1,019, 8,500 ± 780, 10,383 ± 1,227, 11,466 ± 1,039, and 11,087 ± 1,632 μm^2 for the subcutaneous, mesenteric, omental, intrapelvic, and perirenal adipose depot, respectively. Immunohistochemistry using anti-bovine CD172a antibodies was performed to determine the proportion of ATM (the number of CD172a-positive cells per 100 adipocytes, given as a percentage). Expression of *LEP*, *IL6*, and *TNF* was positively associated with adipocyte size, whereas no association could be detected between *ADIPOQ* and *IL10* with the size of the adipocytes. The omental adipose depot was especially infiltrated with ATM (1.92 ± 0.55, 1.10 ± 0.33, and 8.28 ± 2.24% for the subcutaneous, mesenteric, and omental adipose depot, respectively). The proportion of ATM was positively associated with the size of the adipocytes in the omental and mesenteric adipose depot. Expression of *ADIPOQ*, *LEP*, *IL6*, *TNF*, and *IL10* differed among depots, which suggests differences in inflammatory characteristics depending on the anatomical location of depots. In conclusion, the results of the present study confirm the adipose tissue as a potential source of inflammatory mediators and demonstrate ATM infiltration, especially in the omental adipose depot.

Key words: adipokine, adipose tissue, adipose tissue macrophage, mRNA expression

INTRODUCTION

Important physiological changes occur during the transition period of dairy cows, being the last 3 wk before calving and the first 3 wk of lactation (Drackley, 1999). Despite much progress made regarding the metabolism of dairy cows during the transition period, 30 to 50% of the dairy cows suffer from 1 or more health disorders in the transition period (LeBlanc, 2010). Multiple risk factors have been identified in the development of transition cow disorders, among which excessive mobilization of body fat is well characterized (Drackley, 1999; McArt et al., 2013; Roche et al., 2013). Recent research indicates that a dysregulation of inflammatory processes in the periparturient dairy cow

Received September 1, 2017.
Accepted February 28, 2018.
[1]These authors contributed equally to this work.
[2]Corresponding author: dekoste2@msu.edu

may contribute to the development of infectious and metabolic disorders (Trevisi et al., 2012; Bradford et al., 2015; Sordillo, 2016). Different underlying reasons exist for the dysregulation of inflammatory processes, which have been reviewed by Sordillo and Raphael (2013) and Bradford et al. (2015).

Adipose tissue is considered to have a causative role in the dysregulation of inflammatory processes (Brackley et al., 2005; Loor et al., 2007; Contreras et al., 2017). In humans, obesity is associated with a chronic proinflammatory state, known as a metabolically triggered inflammation or metaflammation (Hotamisligil, 2006). In the obese state, macrophages infiltrate the adipose tissue, triggering an inflammatory response that is considered a risk factor for the development of insulin resistance, type-2 diabetes mellitus, and cardiovascular disease (Després and Lemieux, 2006; Cornier et al., 2008). The excessive accumulation of abdominal or visceral fat is considered to be especially detrimental for humans, due to metabolic and functional differences between the subcutaneous and abdominal adipose depot (Després and Lemieux, 2006). Large visceral adipocytes demonstrate a higher lipolytic activity, lower insulin sensitivity, and a dysregulated production of adipokines characterized by higher pro- and lower anti-inflammatory levels of adipokines (Després and Lemieux, 2006). Over-conditioned dairy cows are known to be at an increased risk for the development of different transition problems; this is, in the first place, due to excessive release of free fatty acids from the adipose tissue in the immediate postpartum period (Roche et al., 2009). However, it is not known if high lipolysis rates in over-conditioned cows leads to inflammatory responses that trigger adipose tissue macrophage (**ATM**) infiltration and dysregulated production of adipokines. Recent research indicates that overfeeding energy in nonpregnant and nonlactating dairy cows changes the adipose transcriptome, characterized by increased expression of lipogenic genes and increased expression of genes involved in inflammatory pathways. The transcriptome of mesenteric adipose tissue was altered by the energy level compared with subcutaneous adipose tissue (Moisá et al., 2017).

The aim of the present study was to determine the number of ATM and the mRNA expression of adipokines [adiponectin (*ADIPOQ*), leptin (*LEP*), interleukin 6 (*IL6*), tumor necrosis factor (*TNF*), interleukin 10 (*IL10*)] in different adipose depots from cows with a variable BCS at the end of the dry period. We hypothesized that the number of ATM and the expression of the different adipokines depend on the size of the adipocytes and differ between the different adipose depots.

MATERIALS AND METHODS

All experimental procedures were approved by the ethical committee of the Faculty of Veterinary Medicine (EC2010/149, Ghent University, Belgium).

Study Design

Ten clinically healthy, pregnant Holstein Friesian dairy cows (upcoming parity 2 to 5, age 3.9 ± 1.4 yr, milk yield in the previous lactation 8,750 ± 1,446 kg; mean ± SD) were selected at the beginning of the dry period based on BCS according to the scale of Edmonson et al. (1989) to ensure an equal spread in BCS from normal conditioned (2.5–3.5; n = 5) to over-conditioned (3.75–5; n = 5). During the dry period (starting approximately 7 wk before the expected parturition date), animals were weekly monitored by assessment of their BCS and all animals received similar diets to meet but not exceed requirements. A detailed description of the study design and the diets can be found in De Koster et al. (2015).

Cows were euthanized 10 to 13 d before the expected parturition date (actual days of pregnancy at the moment of euthanasia was 269 ± 5 d) at the Department of Morphology (Faculty of Veterinary Medicine, Ghent University, Belgium). Cows were stunned with a captive bolt gun and exsanguinated by transecting the carotid arteries and the jugular veins. Body weight of the animals the day before euthanasia was 717 ± 77 kg. Immediately after euthanasia, adipose tissue samples were collected from the subcutaneous, omental, mesenteric, perirenal, and intrapelvic adipose depots. The weight of the different adipose depots was determined and is described in De Koster et al. (2015). Subcutaneous adipose tissue samples were taken from the adipose tissue located in the fossa ischiorectalis. Omental adipose tissue samples were taken from the omentum majus at the right side at the level of the pylorus, halfway between the cranial and caudal rim of the omentum. Mesenteric adipose tissue samples were taken 15 cm proximal from the jejunum. Perirenal adipose tissue samples were taken from the caudal end of the right kidney. Samples for mRNA expression were immediately snap frozen in liquid nitrogen and stored at −80°C until processing. Samples for histology were fixed in 4% buffered formaldehyde (pH 7.4) at room temperature for 24 h, subsequently dehydrated in a tissue processor (Microm STP 420D, Prosan, Merelbeke, Belgium) and embedded in paraffin blocks using an embedding station (Microm EC 350–1 and Microm EC 350–2, Prosan).