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Changes of muscle fibre profile and fat cell size around first parturition in cows differing in lactation performance

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ABSTRACT

The presented study investigated changes of fat cell and muscle fibre size, and muscle fibre type composition around first parturition in cows with a combined beef and dairy genetic background. In contrast to high-merit dairy cows, these cows can serve as a model for beef cows because of the very low milk production level. Fifty heifers used in this experiment were F_2 offspring originating from mating Charolais bulls to German Holstein cows and a following intercross of F_1 individuals. They were assigned to 3 groups according to their lactation performance, namely high lactating (HL), low lactating long (LLL; duration of first lactation at least 100 days), and low lactation short (LLS; duration of first lactation shorter than 100 days). Biopsy samples were taken from semitendinosus muscle (MST) and subcutaneous fat (SCF) at three time points, 10 days before first parturition, 30 and 100 days after calving. These samples were investigated by histology and computer image analysis for muscle fibre profile, as well as intramuscular and subcutaneous fat cell size to estimate the impact of lactation on body reserves. Most cows continued growth and gained weight during lactation. Continuous tissue accretion was indicated on the cellular level by an increase in intramuscular fat cell size. Subcutaneous fat cells were larger in low lactating cows with a short lactation period. There were no morphological signs of fat mobilization from fat cells. There was also no protein mobilization indicated by changes in muscle fibre size. However, the increase in percentage of fast, glycolytic muscle fibres indicated alterations in muscle metabolism during transition from late pregnancy to early lactation. Cows remained obviously in a positive energy balance in this study and continued tissue accretion during lactation. This was reflected on the cellular level in skeletal muscle and subcutaneous fat.

1. Introduction

Dairy cows are often not able to compensate the increased energy demands of the foetus and for mammary gland development and milk synthesis by increased energy intake during transition from late pregnancy to early lactation, thus leading to a negative energy balance (NEB) which is intensified by a marked decrease in dry matter intake immediately

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before calving (Buckley et al., 2003; Duffield, 2000; Kessel et al., 2008; Kim and Suh, 2003; Nebel and McGilliard, 1993; Schröder and Staufenbiel, 2006). Body reserves, such as fat depots and muscle protein, are mobilized to compensate this deficit in energy intake during early stages of lactation (Grummer and Rastani, 2003; Hattan et al., 2001; Nebel and McGilliard, 1993). For most cows, breakdown of fat is sufficient to maintaining energy balance during peak lactation, but if cows remain in a state of NEB, other tissues like muscle must be mobilized to meet the energy requirements (Gray, 2008). Thus, associations between differences in milk production and body composition are obvious (Hammon et al., 2010). Furthermore, these differences are based on cellular changes. The relationship of fat cell size to body lipid mass is quite strong in lactating cattle (McNamara, 2000). In adult or almost adult ruminants, the majority of changes in magnitude of the subcutaneous adipose depot is due to changes in mean cell size and can therefore be considered as a measure for fat storage or mobilization (Waltner et al., 1994). The study of Waltner et al. (1994) showed that fat cell diameter is sensitive to alterations in rate and stage of lactation. Following peak lactation as animals started to regain lipid reserves, it can be observed that smaller cells fill up with lipid and the volume of cells increases during later lactation (Smith and McNamara, 1990).

Indicators of skeletal muscle breakdown and increased degradation of skeletal muscle protein can be detected (Chibisa et al., 2008; Gray, 2008), if NEB during lactation persists. Since muscle fibre type profile is considered as an indicator for muscle metabolism, changes in muscle fibre size or type profile can be considered as a measure for protein mobilization (Lefaucheur, 2010).

These processes were intensively investigated in dairy cows, whereas little is known whether they are also important in beef cows. Milk yield of the dam strongly influences average daily gain of the calf and determines the weaning weight (Jeffery et al., 1971), which is of economic importance in beef cow herds.

In our earlier study (Hamada et al., 2012), we investigated the consequences of different lactation performance on carcass composition, muscle structure, and meat quality in 50 F_2 generation cows of a Charolais × German Holstein crossbred experiment (Kühn et al., 2002). The results were obtained after slaughter during the second lactation, 30 days after parturition, and revealed no significant differences in cellular traits between groups of cows with low or high lactation performance. The current investigation was undertaken to study changes of muscle structure and fat cell size of the same cows during the first lactation. The hypothesis was that metabolic adaptations during transition from late pregnancy to early lactation are reflected by structural changes in subcutaneous and intramuscular fat cells as well as in muscle fibres of primiparous cows dependent upon lactation performance. We analysed muscle biopsy samples which were taken 10 days before and 30 and 100 days after calving from cows with a combined beef and dairy genetic background and high variation in lactation performance.

2. Material and methods

2.1. Animals and samples

Fifty cows from a F_2 resource population generated from the founder breeds Charolais and German Holstein (Kühn et al., 2002) were used in this study. Details were described elsewhere (Hamada et al., 2012). Briefly, heifers were reared in a free-stall barn and were inseminated at 18 months of age with semen from German Holstein sires. Heifers calved after 278 ± 4 d of pregnancy and were milked after parturition twice daily in a milking parlour. Feeding details are described by Hammon et al. (2007, 2010). Before parturition, heifers were fed grass silage ad libitum (235 g of CP/kg of DM and 6.75 MJ of NEL/kg of DM), 1 kg of concentrates (150 g of CP/kg of DM and 7 MJ of NEL/kg of DM; mainly barley, sugarbeet pulp, and extracted soybean meal; RM 2007, Vollkraft, Güstrow, Germany) and 300 g of a mineral and vitamin mixture (Salvana 9237, Salvana, Sparrieshoop, Germany). After parturition, heifers received grass silage ad libitum, 3 kg of concentrates, and 300 g of minerals. When milk yield exceeded 15 kg of milk/d, heifers were fed 1 kg of concentrates for every 2 kg of extra milk.

The experimental procedures were carried out according to the animal care guidelines and were approved by the relevant authorities of the State *Mecklenburg-Vorpommern, Germany*.

Cows were assigned to 3 groups according to their milk yield or lactation performance during the first lactation. The groups were named high lactating (HL, $n = 16$), low lactating long (LLL; duration of first lactation at least 100 days; $n = 19$), and low lactation short (LLS; duration of first lactation shorter than 100 days; $n = 15$). Each group contained members of 3 families (A, B, C—offspring of 3 P0 Charolais bulls). Biopsy samples were taken from *M. semitendinosus* (MST) and subcutaneous fat (SCF) at three time points, 10 days before, 30 days after, and 100 days after first parturition using the established shot biopsy technique (Bellmann et al., 2004; Schöberlein, 1989; Wegner and Schöberlein, 1984). The region, where the biopsy was taken, was prepared in accordance to surgical, aseptic standards of operation. This included clipping of the hair and subsequent wet shaving together with cleaning of the area and aseptic treatment with a disinfectant. Wound treatment included a dressing of the wound with an antibiotic spray to avoid infections (Albrecht et al., 2011; Bellmann et al., 2004). Samples of 3 to 5 g, including muscle, SCF, and skin, were harvested with a 6 mm cannula. Muscle and SCF were snap frozen in liquid nitrogen, and stored at −70 °C until use.

The weight of cows was recorded immediately before biopsy. Backfat thickness was measured using ultrasound according to Schröder and Staufenbiel (2006).

2.2. Histology and image analysis

For fat cell size determination (either intramuscular fat [IMF] or SCF), frozen samples were cryosectioned (10 or 20 μm thick, muscle and adipose tissue, respectively) using a Leica CM3050 S (Leica, Bensheim, Germany) cryostat microtome. Sections of SCF were stained with eosin and muscle sections including IMF with haematoxylin and eosin according to Böck (1989). Additionally, serial sections of muscles were stained with Oil Red-O as a control to ensure detection of IMF cells.

Fat cell size was measured using the interactive measurement module of an image analysis system equipped with a Nikon Microphot SA microscope (Nikon Instruments Europe