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Effect of increasing body condition on oxidative stress and mitochondrial biogenesis in subcutaneous adipose tissue depot of nonlactating dairy cows

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ABSTRACT

With the onset of lactation, dairy cows with a body condition score >3.5 are sensitive to oxidative stress and metabolic disorders. Adipose tissue (AT) can adapt to varying metabolic demands and energy requirements by the plasticity of its size during lactation. In AT, angiogenesis is necessary to guarantee sufficient oxygen and nutrient supply for adipocytes. Cellular energy metabolism is reflected mainly by mitochondria, which can be quantified by the mitochondrial DNA copy number per cell. In the present study, we aimed to investigate the effect of overconditioning on angiogenesis and mitochondrial biogenesis in AT of nonlactating cows, irrespective of the physiological influences of lactation and pregnancy. Eight nonpregnant, nonlactating cows received a ration of increasing energy density for 15 wk, during which body weight and body condition increased substantially. Subcutaneous AT was biopsied every 8 wk, and blood was sampled monthly. The blood concentrations of indicators of oxidative stress increased continuously throughout the experimental period, possibly damaging mitochondrial DNA. Concomitantly, HIF-1α, a major marker for hypoxia, increased until wk 8, indicating insufficient angiogenesis in the rapidly expanding AT. Based on the observation that the number of apoptotic cells decreased with increasing hypoxia, the increasing mitochondrial DNA copy numbers might compensate for the hypoxia, reinforcing the production of oxidative stressors. Key transcription factors of mitochondrial biogenesis were largely unaffected. Thus, increased oxidative stress does not impair mitochondrial DNA.

Key words: adipose tissue, dairy cow, mitochondrial biogenesis, oxidative stress

INTRODUCTION

After calving, most cows undergo a phase of negative energy balance, in which the energy demand for milk synthesis is not covered by voluntary feed intake. To meet the increased energy demands, cows mobilize body reserves predominantly from adipose tissue (**AT**). Over the course of lactation, milk synthesis decreases and energy depots are refilled, leading to a positive energy balance (Drackley et al., 2005). In early lactation, overconditioned cows mobilize more body reserves than thin cows (Treacher et al., 1986) and are more susceptible to metabolic disorders, as well as health and reproduction problems (Gearhart et al., 1990; Goff and Horst, 1997; Roche et al., 2009).

During lactation, AT actively adapts to metabolic needs by mobilizing energy stores (lipolysis) and refilling fat depots (lipogenesis). In obese animals, the blood supply in AT is adapted to dynamic cellular processes via angiogenesis, to provide sufficient nutrients and oxygen for the cells, support fatty acid and glycerol release (Lu et al., 2012; Elias et al., 2013; Lemoine et al., 2013), or both. Vascular endothelial growth factor A (**VEGF**) is the key regulator of vasculogenesis and angiogenesis (Tam et al., 2009), stimulating the migration, permeability, proliferation, and survival of endothelial cells (Ferrara and Alitalo, 1999; Shibuya, 2001). The angiogenic and mitogenic effects of VEGF are mediated mainly through tyrosine kinase receptor VEGF-R2 (Terman et al., 1991; Shalaby et al., 1995). In AT, VEGF is thought to be involved in energy metabolism (Lu et al., 2012), and its increased expression protects against the negative consequences of diet-induced obesity and metabolic dysfunction (Elias et al., 2013).

Rapid expansion of AT and adipocyte sizes leads to an increase in intercapillary distance, resulting

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in decreased blood flow and reduced oxygen supply (Cao, 2013). In obese humans and mice, insufficient oxygen supply might cause local hypoxia. In response to hypoxia, AT produces hypoxia-inducible-factor-1α (**HIF-1α**), a transcription factor that in turn induces angiogenic growth factors (Scannell et al., 1995; Mason et al., 2007; Lemoine et al., 2013). Moreover, upregulation of HIF-1α can lead to inflammation (Ye et al., 2007) and cell death in AT (Yin et al., 2009).

In cows with a BCS >3.5 before calving and greater BCS loss after calving, metabolic stress is accompanied by increased oxidative stress (Bernabucci et al., 2005). Oxidative stress derives mainly from an imbalance between the production of reactive oxygen species (**ROS**) by mitochondria and antioxidant defenses that convert ROS to less malign molecules (Sies, 1991; Bernabucci et al., 2005). High concentrations of ROS in periods of increased metabolic demand can damage proteins, lipids, DNA, and the mitochondria themselves (Sawyer and Colucci, 2000; Williams, 2000). Mitochondrial DNA (**mtDNA**) is not protected by proteins such as histones, so it is more susceptible to damage from oxidative stress than nuclear DNA (Richter et al., 1988). Damaged mtDNA can result in a decline of mtRNA transcription and lead to dysfunction of mitochondrial biogenesis (Wallace, 1999).

Mitochondrial biogenesis describes both the proliferation and differentiation of mitochondria (Izquierdo et al., 1995). One of the main markers of mitochondrial proliferation is the mtDNA copy number per cell (Al-Kafaji and Golbahar, 2013). Genes involved in the transcription, regulation, and maintenance of mtDNA, such as nuclear respiratory factors 1 and 2 (*NRF1* and *NRF2*), mitochondrial transcription factor A (*TFAM*), and peroxisome proliferator-activated receptor-γ coactivator (*PGC-1α*; Izquierdo et al., 1995) may change their expression through varying energy supply (Lee et al., 2008).

We hypothesized that overconditioning in cows during positive energy balance leads to local hypoxia in AT due to insufficient angiogenesis. This might change the cellular energy supply and alter the number of mtDNA copies, result in programmed cell death (apoptosis) in AT, or both. Furthermore, oxidative stress might impair the number and function of mitochondria in bovine AT. To describe the local hypoxia and its relation to angiogenesis, we evaluated HIF-1α and pro-angiogenic factors VEGF-A and VEGF-R2. We determined the mtDNA copy numbers per cell and the abundance of genes involved in the transcription, regulation, and maintenance of mtDNA in subcutaneous AT from overconditioned cows. We also assessed the concentrations of advanced oxidation protein products (**AOPP**); lipid peroxidation by measuring thiobarbituric acid reactive substances (**TBARS**); and derivatives of reactive oxygen metabolites (**dROM**) as indicators of oxidative stress, examining their relationship to mtDNA content and mitochondrial biogenesis.

MATERIALS AND METHODS

Experimental Design and Sample Collection

The animal experiment was performed according to European Community regulations and approved by the Lower Saxony State Office for Consumer Protection and Food Safety (LAVES), Germany. The experimental design has been published previously (Dänicke et al., 2014). In brief, 8 nonpregnant, nonlactating German Holstein cows (age 4–6 y) were kept in an open barn and fed solely with crop straw offered ad libitum for 5 mo. Then, at the start of the observation period, the proportion of straw was gradually decreased and the animals were adapted to a high-energy ration by a weekly increase of the proportions of the corn and grass silage mixture from 0 to 40% of DM and concentrate feed from 0 to 60% of DM over 6 wk. This diet was then maintained for a further 9 wk. Body weight (kg) and BCS (according to the 5-point scale by Edmonson et al., 1989) were monitored every 2 wk.

Blood samples from the jugular vein were collected monthly, and subcutaneous AT biopsies were taken from the tailhead region at the beginning of the experiment (0 wk), and at 8 and 15 wk, as described previously (Locher et al., 2015). Tissue samples were immediately snap-frozen in liquid nitrogen and stored at −80°C to isolate DNA and RNA for quantitative PCR (**qPCR**) or were fixed in 4% paraformaldehyde (Roth, Karlsruhe, Germany) for histological evaluation.

Variables Indicative of Oxidative Stress

Oxidative stress was determined in serum by dROM test using *N*,*N*-diethyl-para-phenylendiamine (DEPPD) as a chromogenic substrate (Alberti et al., 2000) with the modifications of Regenhard et al. (2014). The results were expressed as H_2O_2 equivalents.

In plasma, AOPP were determined by modified spectrophotometric methods (Witko-Sarsat et al., 1998; Celi et al., 2011). Different dilutions (6.25 to 100 μ*M*) of Chloramin-T (Sigma-Aldrich, Darmstadt, Germany) in PBS (pH 7.3) were used to generate standard curves, and PBS without Chloramin-T served as a blank. Samples and standards were incubated with 40 μL of pure acetic acid (Roth) for 5 min at room temperature, and 20 μL of potassium iodide (Sigma-Aldrich) was