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mRNA expression of genes involved in fatty acid utilization in skeletal muscle and white adipose tissues of sows during lactation

Robert Ringseis [a], Kristin Heller [b], Holger Kluge [b], Klaus Eder [a,*]

[a] Institute of Animal Nutrition and Nutrition Physiology, Justus-Liebig-Universität Giessen, D-35392 Giessen, Germany
[b] Institute of Agricultural and Nutritional Sciences, Martin-Luther-Universität Halle-Wittenberg. D-06120 Halle, Germany

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ABSTRACT

Rodents are able to lower fatty acid utilization in liver and muscle during lactation in order to spare fatty acids for the production of milk triacylglycerols, an effect which is mediated by a down-regulation of peroxisome proliferator-activated receptor α (PPARα). The present study was performed to investigate whether similar fatty acid sparing effects are developing in lactating sows. We considered PPARα and its target genes involved in fatty acid utilization in biopsy samples from muscle and adipose tissue of lactating compared to non-lactating sows. In muscle, PPARα target genes involved in fatty acid utilization were up-regulated during lactation indicating that the fatty acid utilization in muscle was increased. Activation of PPARα was probably due to increased concentrations of non-esterified fatty acids in plasma observed in the lactating sows. In contrast to muscle, PPARα and its target genes involved in β-oxidation in white adipose tissue were down-regulated in early lactation. Overall, the present study shows that sows, unlike rats, are not able to reduce the fatty acid utilization in muscle in order to spare fatty acids for milk production. However, fatty acid oxidation in adipose tissue is lowered during early lactation, an effect that might be helpful to conserve fatty acids released from adipose tissue for the delivery into other tissues, including mammary gland, via the blood.

1. Introduction

Lactation is a physiological state characterized by a dramatic increase in the energy and nutrient requirement of the organism for milk production. This demand is usually met by a markedly increased food intake and by the utilization of energy stores. In rats, additionally several metabolic adaptations are developing during lactation which aim to conserve energy and metabolic substrates for milk production in the mammary gland (Trayhurn et al., 1982; Williamson, 1986; Dewey, 1997; Smith and Grove, 2002). Down-regulation of proteins involved in fatty acid uptake and oxidation in liver and muscle, effects that help to spare fatty acids for milk production in the mammary gland, contributes to these metabolic adaptations in the lactating rat (Whitelaw and Williamson, 1977; Trayhurn et al., 1982; Pedraza et al., 2000; Pedraza et al., 2001; Xiao et al., 2004a, 2004b). Interestingly, plasma and tissue carnitine concentrations are also reduced in rats during lactation (Gutgesell et al., 2009a). This effect which is due to a down-regulation of hepatic enzymes involved in carnitine synthesis is regarded as an additional means to reduce the rate of fatty acid oxidation. As all the proteins involved in fatty acid uptake and oxidation and carnitine synthesis are transcriptionally regulated by peroxisome proliferator-activated receptor α (PPARα) (Mandard et al., 2004), we have recently postulated that these metabolic adaptations during lactation are induced by a down-regulation of that transcription factor. PPARα is a ligand-activated transcription factor, which is abundantly expressed in tissues with high rates of fatty acid oxidation, such as liver and skeletal muscle, and its physiologic role lies in the mediation of metabolic responses to fasting (Schoonjans et al., 1997; Leone et al., 1999; Mandard et al., 2004). Upon activation by either non-esterified fatty acids (NEFA) released from adipose tissue and taken up into tissues or exogenous ligands (diet-derived fatty acids or fibrates), PPARα up-regulates genes involved in all aspects of fatty acid catabolism including cellular fatty acid uptake and transport, mitochondrial and peroxisomal fatty acid oxidation as well as ketogenesis (Mandard et al., 2004). Indeed, we found that the down-regulation of genes involved in fatty acid uptake and oxidation as well as carnitine synthesis during lactation is mediated by suppression of PPARα in liver and muscle (Gutgesell et al., 2009b). This means that down-regulation of PPARα in muscle and liver plays an important role for the metabolic adaptations occurring during lactation in rats. In line with this suggestion, it has been shown that activation of PPARα during lactation by feeding an oxidized fat disturbs the metabolic adaptation which in turn leads to a reduced concentration of fat in the milk and to an impairment of the development of the suckling pups

Abbreviations: ACO, Acyl-CoA oxidase; CPT-1, Carnitine palmitoyl transferase-1; FAT, Fatty acid translocase; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; LCAD, Long-chain acyl-CoA dehydrogenase; LPL, Lipoprotein lipase; NEFA, Non-esterified fatty acids; OCTN2, Novel organic cation transporter-2; PPARα, Peroxisome proliferator-activated receptor α; TAG, Triacylglycerols.

* Corresponding author. Tel.: +49 641 9939230; fax: +49 641 9939239.
E-mail address: Klaus.eder@ernaehrung.uni-giessen.de (K. Eder).

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(Ringseis et al., 2007). It is likely that the down-regulation of PPARα in tissues of rats during lactation is due to a decrease of the concentrations of NEFA in plasma. In rats, plasma concentration of NEFA during lactation is decreasing, in spite of a considerable release of NEFA from white adipose tissue by hormone-sensitive lipase, as a consequence of a strong up-regulation of fatty acid transporters in the mammary gland and the resulting strongly increased uptake of NEFA from the blood into the mammary gland (Pedraza et al., 2000; Xiao et al., 2004a; Gutgesell et al., 2009b). In rats, moreover, plasma triacylglycerol (TAG) concentration is decreased during lactation, due to an increased expression of lipoprotein lipase (LPL) in the mammary gland (Gutgesell et al., 2009a).

In pigs, expression of PPARα has been also demonstrated and it has been suggested that it has a similar function as in humans and rodents (Cheon et al., 2005; Luci et al., 2007). Regarding expression of PPARα, there is, however, a different tissue profile between pigs and rats. Expression of PPARα in the liver is much lower in pigs than in rodents (Cheon et al., 2005; Luci et al., 2007). In contrast, pigs have a considerable expression of PPARα in the white adipose tissue (Ding et al., 2000) while in rats it is nearly completely absent in adipose tissue (Escher et al., 2001). Pigs have also a remarkably high energy demand during lactation for milk production, but it has not yet been investigated whether pigs show similar metabolic adaptations during lactation as rats. Therefore, the aim of this study was to find out whether pigs show similar metabolic adaptations during lactation as rats. For that purpose, we determined the expression of PPARα and its target genes in skeletal muscle and white adipose of lactating sows compared to sows whose litters were removed at parturition which serve as non-lactating controls. To obtain information about alterations in the uptake of fatty acids into the mammary gland during lactation, we also determined expression of the fatty acid transporter fatty acid translocase (FAT) and LPL in the mammary gland as well as NEFA and TAG concentrations in plasma.

2. Materials and methods

2.1. Animals and housing

Sixteen crossbred sows (*Sus scrofa*) (German land race × Large white) with an average body weight of 255 (±17, SD) kg and a parity number of three were used for this study. The sows were artificially inseminated with sperm from Pietrain boars. Until day 30 of pregnancy, all the sows were kept in single crates. Thirteen out of the sixteen sows conceived. Before parturition the pregnant sows were randomly assigned to 2 groups of six and seven animals, respectively. In one group, all pups were removed from the sow at the second day after parturition ("non-lactating"). This group of sows served as non-lactating control group. In the other group of sows, litters were adjusted to 15 piglets per sow ("lactating"). In that group, the piglets were suckled by their mothers for a period of 26 days. All animal procedures described followed established guidelines for the care and handling of laboratory animals and were approved by the regional council of Saxony-Anhalt.

2.2. Diets and feeding

Two commercial sow diets were used. The first one, fed from the start of the experiment until day 110 of pregnancy, was a commercially available diet for *ad libitum* consumption for gestating sows (Deuka SF-Satt gekörnt, Roth GmbH & Co Agrarhandel, Kirchhain, Germany) which contained (per kg diet) 9.0 MJ metabolizable energy, 130 g crude protein, 22 g crude fat, 145 g crude fibre and 60 g crude ash. The second diet, fed from day 110 of pregnancy until weaning, was a commercially available diet for lactating sows (Deuka Lactosan Mehl, Roth GmbH) which contained (per kg diet) 13.4 MJ metabolizable energy, 175 g crude protein, 50 g crude fat, 50 g crude fibre and 59 g crude ash. From the beginning of the experiment until day 30 of pregnancy the sows were offered 3.0 kg of gestation diet per day; from day 30 to day 110 the diet was offered for *ad libitum* consumption. From day 110 to farrowing each sow was fed 2.5 kg of lactation diet. On the day of farrowing the sows were fed 1.5 kg/day. In the group of sows with litters, the amount of diet was then successively increased (3 kg/day on day 1 and day 2 of lactation; 4.5 kg/d on day 3 and day 4 of lactation; *ad libitum* consumption from day 5 of lactation to weaning). In the group of sows without litters, the animals received the lactation diet *ad libitum*. For technical reason, it was not possible to record the food intake of the sows. Water was provided by nipple drinking systems.

2.3. Sample collection

At days 7 and 17 after parturition, biopsy samples from m. *longissimus dorsi* and subcutaneous white adipose tissue (over the m. *longissimus dorsi*) and blood samples (from *vena jugularis*) were taken in the morning after the first meal from both groups of sows. Blood was collected into heparinised polyethylene tubes (Sarstedt, Nürnberg, Germany). Plasma was obtained by centrifugation of the blood (1100 g; 10 min; 4 °C) and stored at −20 °C. Muscle and white adipose tissue biopsy samples were immediately shock frozen with liquid nitrogen and stored at −80 °C pending analysis. At day 26, all the sows were slaughtered in a local abattoir. Mammary glands from left and right ventral surfaces were removed. Two mammary glands, one from the left side and one from the right side which represented the average size were selected for molecular biological analysis. Those mammary glands were prepared by removing skin and adipose tissue with a scalpel.

2.4. Lipid analysis

Plasma NEFA and TAG concentrations were measured using enzymatic reagent kits obtained from Wako Chemicals (Neuss, Germany, Ref. 99975406) and Merck Eurolab (Darmstadt, Germany, Ref. 113009990314) according to the manufacturers' protocol.

2.5. Carnitine analysis

Concentrations of free carnitine and carnitine esters in plasma were determined by tandem mass spectrometry using deuterated carnitine-d3 (Larodane Fine Chemicals, Malmö, Sweden) as internal standard (Hirche et al., 2009). The concentration of total carnitine was calculated from the concentrations of free carnitine and carnitine esters (acetyl, propionyl).

2.6. RNA isolation and real-time RT-PCR

For the determination of mRNA expression levels total RNA was isolated from muscle, subcutaneous adipose tissue and mammary gland samples using TrizolTM reagent (Invitrogen, Karlsruhe, Germany) according to the manufacturer's protocol. RNA concentration and purity were estimated from the optical density at 260 and 280 nm, respectively. A total of 1.2 μg of total RNA was subjected to cDNA synthesis using M-MuLV Reverse Transcriptase (MBI Fermentas, St. Leon-Rot, Germany). For determination of mRNA expression levels real-time detection RT-PCR using the Rotorgene 2000 system (Corbett Research, Mortlake, Australia) was applied. A total of 2 μL cDNA templates were amplified in 100 μL Rotorgene PCR tubes in a final volume of 20 μL containing 500 μmol/L dNTP (Roth), 3.5 mmol/L $MgCl_2$, 1.25 U GoTaq® Flexi DNA Polymerase, 4 μL 5× buffer (all from Promega, Mannheim, Germany), 0.5 μL 10× Sybr Green I (Sigma-Aldrich, Taufkirchen, Germany), and 26.7 pmol of each primer pair. The PCR protocol was composed of an initial denaturation at 95 °C for 3 min and 20–35 cycles of amplification comprising denaturation at