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Lactation-related changes in tissue expression of *PEDF* in dairy cows

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ABSTRACT

Pigment epithelium-derived factor (PEDF) is evolving as metabolic regulatory protein. Albeit mostly considered in only pathological conditions related to excess energy intake resulting in obesity and insulin resistance, PEDF is likely to be involved in other physiological processes such as the homeorhetic adaptation of metabolism to lactation. We aimed to characterize the expression of PEDF and its association to the concomitant mobilization of body reserves during lactation in nonobese subjects. This mobilization is particularly distinct in dairy cows, and we therefore assessed the mRNA expression of PEDF and its putative receptors in different tissues in 2 trials with dairy cows fed with or without conjugated linoleic acids (CLAs). Conjugated linoleic acids depress milk fat synthesis and may thus reduce the drain of energy via milk. In pluriparous cows, the serum PEDF concentrations and the mRNA abundance in subcutaneous adipose tissue (scAT), as well as the hepatic and scAT mRNA abundance of the putative receptors, adipose triglyceride lipase, and laminin receptor 1, changed over time of sampling (day −21 until day 252 relative to calving). Conjugated linoleic acid treatment was associated with reduced PEDF concentrations in serum and lower PEDF mRNA abundance in scAT on day 21 postpartum. Comparing different tissues from primiparous cows, PEDF mRNA was highest in the liver, followed by scAT, visceral adipose tissue (AT), and mammary gland, and lowest in the muscle. Significant changes in PEDF expression with time of sampling were limited to AT in primiparous and pluriparous cows. Our data support a regulatory role for PEDF. The similarities between the time course of the serum concentrations of PEDF and its mRNA abundance in scAT may point to a regulatory role for AT rather than the liver for PEDF in dairy cows.

1. Introduction

Pigment epithelium-derived factor (PEDF), a 50-kDa secreted glycoprotein first purified from conditioned media of human fetal retinal epithelial cells [1], belongs to the noninhibitory serpin group [2]. It acts as an inhibitor of angiogenesis [3] with neurotrophic [4] and

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immune-modulating properties [5]. PEDF was also recently identified as an adipokine: it is one of the most abundant proteins secreted by human adipocytes [6] and was suggested as a novel marker of body fat mass changes [7]. Furthermore, expression of PEDF in both human myoblasts and myotubes was recently documented and thus PEDF was defined as an adipo-myokine [8]. In human patients with obesity-related diseases, the circulating PEDF concentrations are elevated compared to healthy subjects, are associated with insulin resistance and decrease during diet-induced and gastrectomy-induced weight loss [9,10]. Patatin-like phospholipase domain containing 2, also known as adipose triglyceride lipase (ATGL) and ribosomal protein SA, also known as laminin receptor 1 (LR1) were identified as putative receptors for PEDF [11–13].

PEDF upregulates expression of peroxisome proliferator-activated receptor-γ (PPARγ) and thus suppresses nuclear factor-κB (NFκB)-mediated transcriptional activation [14]. Moreover, PEDF acts as a ligand of PPARα [15] and PPARγ [16], the latter reportedly to a lesser extent [15]. The ligands of PPARγ are structurally diverse and encompass endogenous metabolites, dietary compounds, and synthetic drugs; conjugated linoleic acids (CLA) are a group of polyunsaturated fatty acids with a single pair of conjugated double bonds. The *cis*-9, *trans*11-CLA and the *trans*-10, *cis*-12-CLA isomers that occur naturally as a product of rumination activate PPARγ and α [17]. Commercially available CLA supplements usually contain chemically synthesized *cis*-9, *trans*11-CLA and *trans*-10, *cis*-12-CLA at a ratio of approximately 1:1. From the various benefits claimed for human health, only the body fat and weight reducing effect is evidenced as recently reviewed [18]. The antiobesity effect of CLA is particularly attributed to the *trans*-10, *cis*-12-isomer [19,20], which is also suspected of having prodiabetic effects [21]. In consideration of the CLA-induced decrease in body fat and the positive association of circulating PEDF and adiposity, we hypothesized that supplementation with CLA will decrease PEDF expression in adipose tissue (AT). Based on reports about a divergent response of different visceral (vc) and subcutaneous (sc) fat depots toward CLA [22], and the particular relation of circulating PEDF with vc adiposity [23], we also aimed to compare PEDF expression in not only different fat depots but also in other metabolically relevant tissues. Moreover, the current knowledge about PEDF as an adipokine is largely limited to its relationship with body weight and body fat content in obese patients, whereas physiological changes in nonobese states await characterization.

Most mammals undergo significant changes in body weight and body composition during pregnancy and lactation to cover the energetic costs for fetal growth and for milk. Voluntary feed intake is not increasing as fast as milk production [24], and thus, a cow with 700 kg body weight may mobilize about 54 kg of body fat and 21 kg of body protein during the first 5 weeks of lactation [25]. Dairy cows are thus particularly suitable to study lactation-driven body fat loss. The objectives of our studies were to characterize *PEDF* mRNA expression in different fat depots and also in other tissues and PEDF protein concentrations in serum of dairy cows receiving dietary CLA or a control fat supplement during lactation. In addition, aiming to confirm adipocytes as a *PEDF* producing cell type of AT, we quantified *PEDF* mRNA in differentiating bovine preadipocytes.

2. Material and methods

2.1. Animals, diets, and treatments

The experiments and the treatments of the cows (*Bos taurus*) were approved by the competent authority, the Lower Saxony State Office for Consumer Protection and Food Safety (LAVES, file no. 33.11.42502–04-071/07, Oldenburg, Germany). The regulations of the German Animal Welfare Act (TierSchG) in its respective current edition were met. All animals were housed at the experimental station of the Friedrich Loeffler Institute, Federal Research Institute for Animal Health, Braunschweig, Germany.

In trial 1, 21 pluriparous Holstein cows with mean body condition score (BCS) 3.1 (1–5 scale; where 1 is lean and 5 obese) were fed ad libitum over the entire experimental period based on the recommendations of the German Society of Nutrition Physiology [26]. From day 1 to day 182 postpartum, the animals received either 100 g/d CLA (Lutrell Pure, BASF, Ludwigshafen, Germany, n = 10) or an isoenergetic control fat supplement 100 g/d (Silafat, BASF, n = 11). In the CLA group, the animals consumed 7.6 g/d each of the *trans*-10, *cis*-12 and *cis*-9, *trans*-11 CLA isomers, calculated, based on the analyzed content in the concentrate [27]. In the control fat supplement, these isomers were substituted by stearic acid. Liver and subcutaneous adipose tissue (scAT) (at the tail head) were biopsied on days −21, 1, 21, 70, 105, 182, 196, 224, and 252 relative to parturition as described previously [28]. For gene expression analyses, all sampling dates from the control group and days −21, 21, 105, 196, and 252 of the CLA group were selected. Blood samples obtained via jugular venipuncture on days −21, 21, 49, 105, 196, and 252 relative to parturition from both the control and the CLA group were centrifuged (10 min, 3000 × *g*, 4°C), and serum and heparin plasma were stored at −80°C until analyzed.

In trial 2, 25 primiparous Holstein cows with an average age at first calving of 23 ± 0.2 mo and a mean BCS of 3.0 were fed analogous to trial 1. Five animals were slaughtered on day 1 postpartum (average age at parturition: 23 mo), and the remaining animals (n = 20) were randomly allocated to either 100 g/d of the control fat (Silafat, BASF) or the CLA supplement (Lutrell Pure, BASF) from day 1 until slaughter at days 42 and 105 postpartum (n = 5 per group and date). Further information about the experiment and the effects of CLA treatment on body composition is provided in detail by von Soosten et al [29]. Samples from three visceral adipose tissue (vcAT) depots (omental, mesenterial, and retroperitoneal), three scAT depots (tail head, withers, and sternum), liver, semitendinosus muscle, pancreas, and mammary gland parenchyma were taken immediately after slaughter.

In trial 3, scAT around the sternum was obtained from 3 additional Holstein dairy cows at slaughter. Tissue treatment, cell separation, culture, and differentiation conditions are described in detail by Hosseini et al [30]. Isolated stromal vascular cells of the 3 cows were pooled and