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The effect of long term under- and over-feeding on the expression of genes related to lipid metabolism in the mammary tissue of goats

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ABSTRACT

Fat synthesis is under the control of a large number of genes whose nutritional regulation is still poorly documented. In this study, we examined the effect of long term under- and over-feeding on the expression of genes (acetyl-CoA carboxylase: ACACA, fatty acid synthetase: FASN, lipoprotein lipase: LPL, stearoyl-Co A desaturase 1: SCD1, peroxisome proliferator activated receptor G2: PPARG2, sterol regulatory element binding protein-1c: SREBP-1c and hormone sensitive lipase HSL) related to FA metabolism in goat mammary tissue (MT). Twenty four lactating goats were divided into three homogenous sub-groups and fed the same ratio in quantities covering 70% (underfeeding), 100% (control) and 130% (overfeeding) of their energy and crude protein requirements. The results showed a significant reduction on mRNA of ACACA, FASN, LPL, SCD1 and HSL in the MT of underfed goats, compared with the respective overfed. A numerical decrease on the mRNA level of PPARG2 and SREBP-1c in the MT of the underfed goats, compared with the respective controls and the overfed was also observed. In conclusion, the negative, compared to positive, energy balance in goats down regulates the ACACA, FASN, LPL, SCD1 and HSL expressions in their MT which indicates that the decrease in nutrient availability may lead to lower rates of lipid synthesis.

1. Introduction

Milk fat is one of the most complex natural fat that consist of approximately more than 400 different fatty acids (FA) (Shingfield et al., 2013). These FA originate from two sources: the *de novo* synthesis in the mammary tissue (MT) (ca. 40%) and the uptake from circulation of preformed FA in blood (ca. 60%) (Cozma et al., 2013). More specifically, MT: synthesize short- and medium-chain FA using acetate and 3-hydroxybutyrate in the present of two key enzymes, acetyl-Co A carboxylase (ACACA) and fatty acid synthatase (FASN), and uptake FA from the plasma with the involvement of lipoprotein lipase (LPL) (Harvatine et al., 2009; Shingfield et al., 2010). These FA maybe further desaturated by the stearoyl-Co A desaturase 1 (SCD1) but not elongated. In addition, the process of the FA biosynthesis is controlled also by transcription factors such as the isoform c of the sterol regulatory element binding protein-1 (SREBP-1c), which is involved in triglycerides synthesis (Rudolph et al., 2010; Ma and Corl, 2012; Xu et al., 2012), and the isoform G2 of the proliferator activated receptor G (PPARG2), which alters lipogenic genes networks in goats mammary epithelial cells (Shi et al., 2013; 2014). Further to that, recent evidence also confirms the involvement

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of hormone sensitive lipase (HSL) gene on lipid metabolism in goats mammary epithelial cells (Lin et al., 2013).

However, despite these genes involvement on milk FA metabolism, little is known concerning the nutritional regulation of these genes in goats MT. Until now the few nutritional studies, which summarized in a recent review by Bernard et al. (2013), and been done in goats MT, have used lipids supplements as dietary effect, in order to considered possible molecular mechanism. However, to the best of our knowledge, despite the fact that small ruminants, particularly in the Mediterranean basin, face periods with fed shortage/surplus the effect of feeding level on MT genes expression, have not been studied previously, except by the expression of FASN in the MT of goats which was measured by Ollier et al. (2007) when the animals fed under normal or 48 h food derivate circumstances. Therefore, the objective of the present study was to determine the effects of long term under- and over-feeding on the expression of genes (ACACA, FASN, LPL, SCD1, PPARG2, SREBP-1c and HSL) related to fatty acids metabolism in goats MT.

2. Materials and methods

Twenty-four 3-year-old dairy goats of native breed at 90–98 days in milk were maintained at the Agricultural University of Athens. All the animals were at the same lactation stage and at the 3rd parity. Housing and care of animals conformed to Ethical Committee guidelines of Faculty of Animal Science. The goats were divided into three homogeneous sub-groups ($n=8$) based on their mean body weight (53.1 ± 2.1 kg) and their mean daily milk yield (0.70 ± 0.083). Each goat of each group was fed individually throughout the experimental period which lasted 60 days. The three sub-groups (treatments) were fed with a diet which covered the 70% (under-feeding), 100% (control), and 130% (over-feeding) of their daily individual energy and crude protein requirements respectively. The quantities of food offered to the animals were adjusted at the 0, 12, 24, 31, 39 and 52 experimental day according to their individual requirements based on their body weight and milk yield.

The diet given to goats consisted of alfalfa hay and concentrates with a forage/concentrate ratio=50/50. The full experimental design has been described with details in the paper of Tsiplakou et al. (2012).

2.1. Sample collection

2.1.1. Milk and blood samples

All the animals were milked twice a day by a milking machine. Individual milk samples were collected from the goats at 30th and 60th experimental day from the beginning of the experiment for chemical composition analysis after mixing the yield from the evening and the morning milking on a percent volume (5%). Blood samples were also taken at the same experimental days for insulin determination from the jugular vein into EDTA-containing tubes and subsequently centrifuged at 2.700*g* for 15 min to separate plasma from the cells.

2.1.2. Mammary tissue samples

Mammary tissue (MT) samples were taken by biopsy on the 30th and 60th experimental day of each dietary treatment after the morning milking. Before the biopsy, the under of the animals was shaved and cleaned and received a local anesthesia. Biopsy samples were taken from the right under at the area of milk synthesis and secretion by using a human Bard® Magnum® Biopsy instrument (BARD, Covington, GA) and a core tissue biopsy needle (14G) (BARD, Covington, GA). The length of the sample notch was about 1.9 cm of up to approximately 15 mg tissue from a depth of 3–5 cm. MT samples were rinsed with 0.9% saline solution inspected to verify tissue homogeneity and snap frozen in liquid nitrogen. After the tissue samples were taken, the site of sampling received a prophylactic treatment with a disinfecting chlorhexidine powder and then covered with plaster by spraying.

2.2. Determination of transcript levels using real-time RT-qPCR assay

Total RNA was isolated from 15 mg of MT using the Trizol reagent (Invitrogen, Paisley, UK) according to the manufacturer protocol. Prior to RT-PCR, the total RNA samples were treated with DNase I (Promega, Madison, WI) at 37 °C for 60 min. The integrity of the RNA was assessed by gel electrophoresis. RNA was quantified with a NanoDrop ND-1000 spectrophotometer, while the purity of RNA was determined by the ratio A260/A280 >1.9. The complete digestion of genomic DNA was confirmed by real-time PCR reaction using our gene specific primers. First-strand cDNA was reverse transcribed from 2 μg of

Table 1
Primers used for real-time RT-Qpcr and the mean PCR efficiency for each gene as calculated by LinRegPCR software (Ramakers et al., 2003) and the mean C_t cycle under control dietary treatment.

Gene	Acc. no.	Forward primer	Reverse primer	PCR efficiency	Mean C_t (control)
ACACA	DQ370054	5′-TCTTTGGCCTACGACGAGATCA-3′	5′-AGGTAAGCCCCAATCCCAATG-3′	0.917	21.9
FASN	DQ915966	5′-TGGTGATGAACGTCTACCGTGA-3′	5′-GGACGTTTATGAAGGCGTGCT-3′	0.957	16.68
LPL	DQ370053	5′-TACCCTAACGGAGGCACTTTCC-3′	5′-TGCAATCACACGGAGAGCTTC-3′	0.965	19.92
SCD1	AF325499	5′-TGTCCACCATGAACCACGTGT-3′	5′-CCACCCCTTAGCTGATGCATT-3′	0.955	18.47
PPARG2	NM_001100921	5′-GGTTGACACAGAGATGCCGTT-3′	5′-TAGAAAGGTCCACGGAGCTGA-3′	0.885	25.45
SREBP-1c	XM_004013336	5′-CGCAAAGCCATCGACTACATC-3′	5′-TGAGCTTCTGGTTGCTGTGCT -3′	0.814	22.11
HSL	NM_001128154	5′- CAAGAGCCTGAAGCTGCATGAC-3′	5′-AGCTCTGGCGTGTCTGTTGTGT-3′	0.905	26.40
RPS9	XM_004015433	5′-TTCGAAGGTAATGCCCTGTTG-3′	5′-TTCATCTTGCCCTCGTCCA-3′	1.000	18.98
UXT	XM_004022128	5′-TCATTGAGCGACTCCAGGAAG-3′	5′- CAGCCCAAATCCACTTGCAT-3′	0.978	23.98