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Effects of fatty acids on S 14 expression in murine preadipocyte cell lines

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sequential ultracentrifugation. ApoHDLs were identified after migration in area-gel at alkaline pH and then transferred on PVDF membranes before sequencing.

Amino acid (AA) composition of goose apoA-I was very similar to that of the chicken. The N-terminal amino acid sequence homology to other avian apoA-I was 91% in the duck and 82% in the chicken. ApoCs-like were described for the first time in avian species and were named apoCa and apoCb. The partial N-terminal sequence of apoCa exhibited a 9 AA motif identical to the one found in mammalian apoC-III. Thus apoCa might be the avian equivalent of apoC-III, which is the main inhibitor of lipoprotein lipase (LPL). Goose apoCb exhibited two isoforms due to differences in the electric charge, Cb₁ and Cb₂. Cb₁ and Cb₂ had the same N-terminal AA sequence and presented no sequence similarity with any other known protein, and especially with apoC-II, which is the main LPL activator in mammals. Cb₁, Cb₂, or both isoforms were detected in 20%, 28% and 52% of the geese, respectively. Their transmission mode was consistent with two segregating alleles from a single concomitantly expressed gene.

The metabolic role of apoCs-like and of their isoforms remains to be established in the goose, especially in relation to susceptibility to liver steatosis.

Effect of the diet on lipid profile of adipose tissue in (fa/fa) obese Zucker rats. R Cantoral, MT Macarulla, MI Torres, MA De Diego, MP Portillo (*Department of Nutrition, Faculty of Pharmacy, University of País Vasco, c/ Marqués de Urquijo s/n, 01006 Vitoria, Spain*).

Genetic obesity observed in fa/fa Zucker rats induces some lipid metabolism modifications. Thus, changes in the concentra-

tion of many fatty acids have been observed in adipose tissue.

The purpose of this work was to investigate if these alterations could be corrected by using different dietary treatments. In this study two factors were considered: the reduction of the energy intake and that of the dietary fat content.

Twenty-eight male Zucker rats were divided into four groups: seven lean rats fed ad libitum (group A), seven obese rats fed ad libitum (group B), seven obese rats fed a 25% energy-restricted diet, which provided 10% of total energy from fat (group C) and seven obese rats fed a 25% energy-restricted diet, which provided 50% of energy from fat (group D). All diets were prepared by using olive oil as fat source.

After 4 weeks, animals were sacrificed by decapitation and subcutaneous adipose tissue was removed. Fatty acid concentrations were measured by gas chromatography. ANOVA test was used for statistical analysis.

Obese rats of group B showed an increase in C 14:0, C 16:0 and C 16:1 and a decrease in C 12:0, C 18:0, C 18:1, C 18:2 and C 18:3. Energy restriction did not allow to correct this fatty acid profile (no statistical difference was found between groups B and C).

In contrast, despite identical energy intake, when the diet provided a high amount of fat, some fatty acid disturbances were completely corrected and others were clearly improved.

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Effects of fatty acids on S 14 expression in murine preadipocyte cell lines. JP Grillasca¹, J Antras-Ferry², H Khiri², C Forest², J Torresani, R Planells. (¹ U 130 and U 38 Inserm, Faculté de médecine, 27, bd J-Moulin, 13000 Marseille; ² Ceremod, 9, rue J-Hetzel, 92190 Meudon, France).

Previous studies have showed that livers of rats fed a diet rich in polyunsaturated fatty acids (FA) presented a fast decrease in mRNA-Spot 14 (S14), which encodes a protein presumed to be implicated in lipogenesis. Here we studied the regulation of S14 gene by FA in adipocytes. To this aim, cultures of fully differentiated 3T3-F442A adipocytes were maintained in 5 mM glucose serum-free medium for 24 h, then supplemented with bovine serumalbumin-FA complexes (mol. ratio, 6/L). To detect even minute variations in the amounts of mRNA-S14, an original quantitative RT-PCR method was designed. This method takes advantage of interspecific polymorphism and normalizes the results with regard to β -actin expression.

Our results show that the amount of mRNA-S14 was weaker in cells cultivated in the presence of unsaturated FA for 24 h, when compared to that of non-supplemented cultures. More specifically, when C18 FA were compared, this decrease was more important if cells were incubated in the presence of linoleate (-66%), than oleate (-50%) or linolenate (-35%). A similar decrease is also shown when cells were maintained in the presence of 1 mM clofibrate for 4 h (-50%). Furthermore, this latter agent is able to totally inhibit the strong induction seen in the presence of 1 μ M retinoic acid (+400% for 4 h). FA and fibrates are thus able to directly down regulate the expression of a lipogenesis-correlated gene.

Study of lipoprotein lipase mRNAs in ovine adipose tissue by quantitative fluorescence polymerase chain reaction. M Bonnet^{1,2}, C Leroux¹, JP Furet¹, P Martin¹, F Le Provost¹, Y Faulconnier², JF Hocquette³, Y Chilliard² (¹ *Laboratoire génétique biochimique et cytogénétique, Inra, 78352 Jouy-en-Josas*; ² *Laboratoire sous-nutrition des ruminants, Inra, Theix*; ³ *Laboratoire croissance et métabolisme*

des herbivores, Inra, Theix, 63122 Ceyrat, France).

To investigate the nutritional regulation of lipoprotein lipase (LPL, a key enzyme for lipogenesis) gene expression, we have developed a strategy of mRNA quantitation based upon fluorescent technology. Reverse transcription of the RNA was followed by polymerase chain reaction amplification (RT-PCR). The relative level of LPL mRNA was determined by coamplifying the targeted transcripts simultaneously with endogenous internal standard (γ -actin or cyclophilin mRNA), arising from genes supposed to be (as house keeping gene) constitutively expressed.

Total RNA was extracted from adipose tissue (AT) from dry, non-pregnant adult ewes either restricted for 7 days (20% of energy maintenance requirement, EMR) or restricted and refed for 14 days (220% of EMR) ($n = 2 + 2$). RNA was reverse transcribed and 28 cycles of amplification were performed with primers specific for LPL, and either γ -actin or cyclophilin. Each of the forward primers were fluorescently labeled and the resulting fluorescent PCR products were analysed with an automated DNA sequencer.

Since the nutritional status was shown to be without any significant effect on the amount of γ -actin and cyclophilin mRNAs, the latter allows to estimate yields at the different steps (RNA extraction, RT and PCR) of the quantitative assay. In contrast, the level of LPL mRNA increased sharply (by 5 to 10-fold according to the endogenous control and the animals) in AT of restricted-refed ewes. This is in agreement with our previous results on LPL activity ($\times 25$) and northern-blot analysis of LPL transcripts [Bonnet et al, *Int J Obesity* (1996), 20, suppl 4, 148].

Taken together, these results suggest a nutritional regulation of LPL gene expression, at least in part at a pretranslational level.