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 μM 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 Leroux [1], JP Furet [1], P Martin [1], F Le Provost [1], Y Faulconnier [2], JF Hocquette [3], Y Chilliard [2] (*[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 (× 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.