Regulation of hormone-sensitive lipase gene expression in 3T3-F442A and BFC-1 adipocytes. E Plee [1], J Grober [2], D Langin [2], C Forest [1] *([1] Ceremod, CNRS UPR 1511, 9, rue Jules-Hetzel, 92190 Meudon; [2] Inserm U 317, Institut Louis-Bugnard, CHU-Rangueil, 31054 Toulouse, France)*

Hormone-sensitive lipase (HSL) catalyzes the rate limiting step in adipocyte lipolysis. As such, it plays a critical role in the control of energetic homeostasis. Short-term hormonal regulation of HSL activity (via phosphorylation-dephosphorylation reactions) is well characterized, whereas not much is known about the control of HSL gene expression. We used two murine adipose cell lines, 3T3-F442A and BFC-1, to develop this latter aspect.

HSL mRNA content was measured by Northern blot using a mouse cDNA fragment. Cells were grown in DMEM containing 10% fetal bovine serum. At confluence, the medium was supplemented with 2×10^{-8} M insulin to favor triglyceride accumulation. Experiments were performed on mature adipocytes (8 days after confluence). Cells were shifted to serum-free, insulin-free medium for 24 h before RNA extraction or HSL assay.

Treatment of 3T3-F442A adipocytes with the β-adrenergic agonist isoproterenol at 1 μM produced a 40% decrease in 18 h. Cyclic AMP (cAMP) reduced HSL mRNA content by a maximum of 60% after 12 h of treatment with 0.5 mM 8-CPT-cAMP. The phorbol ester PMA induced a maximal 50% reduction after 6 h of treatment with 1 μM. The table below summarizes these results. All the effects were unaffected by the protein synthesis inhibitor anisomycin, suggesting that cAMP and PMA actions on HSL gene expression were direct. The decrease in HSL mRNA was followed by a reduction in HSL activity, a direct reflection of protein amount (see table). Similar results were obtained with BFC-1 adipocytes.

Although cAMP and PMA produced a similar reduction in HSL mRNA, the intracellular routes that these agents follow for inducing such an effect, seemed clearly independent because i) after desensitization of the protein kinase-C regulation pathway by a 24 h treatment of the cells with 1 μM PMA, PMA action was abolished whereas cAMP was still fully active; ii) treatment with saturating concentrations of both agents produced an additive effect; iii) the synthetic glucocorticoid dexamethasone had no specific effect on HSL gene expression. However, dexamethasone potentiated cAMP action (see table) without affecting the PMA effect.

cAMP inhibitory action on HSL (mRNA and protein) is unexpected. Indeed, this second messenger of catecholamines is the main activator of HSL activity by phosphorylation. A hypothesis is envisioned that long-term cAMP stimulation induces an adaptative reaction of the adipose cell coun-

Treatment	*% of control*	
	mRNA	*Specific activity*
8-CPT-cAMP, 0.5 mM	41.3 ± 2.74	60.5 ± 6.2
Isoproterenol, 1 μM	60.3 ± 5.6	63.8 ± 11.8
PMA, 1 μM	50.25 ± 4.1	65.3 ± 3.3
Dexamethasone, 0.1 μM	116.6 ± 3.7	84.8 ± 4.1
8-CPT-cAMP+dexa	22.7 ± 2.7	48.8 ± 2.9

Data represent the mean ± SEM of at least three independent experiments.

terregulating HSL content in order to limit fatty acid depletion from the stored triglycerides.

Role of endogenous oleic acid produced by Δ9-desaturase on triacylglycerol secretion in cultured chicken hepatocytes. P Legrand, D Catheline, M Fichot, P Lemarchal *(Laboratoire de biochimie, Inra-Ensa, Rennes, France)*

The relationship between endogenous oleic acid produced by hepatic Δ9-desaturase, and the rate of secretion of very low density lipoprotein (VLDL)-triglycerides was investigated in a primary culture of chicken hepatocytes. This culture system exhibited a high level of Δ9-desaturase activity and triglyceride (TG) secretion, which both peaked in the interval between 30 and 60 h of culture. Oleic acid added to the culture medium of the secreting cells slightly increased the TG secretion rate, but significantly enhanced the total rate of TG synthesis in these cells. The comparison of the fatty acid composition of the secreted and intracellular TG showed an imbalance in the ratio of monoenes and saturated fatty acids, the secreted TG being significantly more unsaturated than the intracellular TG.

Cyclopropenic fatty acids (sterculic and malvalic acids, 0.5 mM, specific inhibitors of fatty acid desaturation) were added to the culture medium 24 h before measurements of Δ9-desaturase activity and TG secretion rate of the hepatocytes. This led to an almost complete suppression of both desaturase activity and TG secretion, measured in the absence of exogenous fatty acid in the culture medium, but did not affect total TG synthesis. However, the addition of 0.5 mM oleic acid to the culture medium of cells treated with cyclopropenic fatty acids was able to restore the TG secretion rate. Linoleic acid was less efficient than oleic acid and palmitic acid was inefficient. Finally, even in the presence of 0.5 mM oleic acid in the culture medium of secreting cells, hepatocytes treated with cyclopropenic fatty acids showed a significant ($P < 0.05$) decrease in TG secretion rate compared to nontreated cells (3.33 ± 0.77 and 6.20 ± 0.95 nmol/mg•h, respectively). Taken together, these results demonstrated that the secretion of TG containing fatty acids produced de novo by chicken hepatocytes is highly dependent on the level of hepatic Δ9-desaturase activity as has been already suggested by previous results in our laboratory. These results suggest that the oversecretion of VLDL-TG in the chicken leading in turn to higher fattening could originate in high desaturation of saturated fatty acids by Δ9-desaturase.

Effect of 17 β-estradiol on hepatic lipid desaturation and secretion in the chicken. D Hermier [1], D Catheline [2], P Legrand [2] *([1] Inra, 37380 Nouzilly; [2] Inra-Ensa, 35042 Rennes cedex, France)*

Hepatic Δ-9 desaturation of de novo synthesized fatty acids (FA) facilitates the synthesis of triglycerides (TG) and their subsequent secretion as very low density lipoproteins (VLDL) [Jeffcoat (1979) *Assays Biochem* 15, 1-36 in the rat; Legrand and Lemarchal (1992) *Comp Biochem Physiol* 102B, 371-375 in the chicken]. In the laying hen, the dramatic increase in the hepatic lipogenesis by estrogens is frequently paralleled with a hepatic steatosis that may compromise egg production and even lead to death. The delicate balance between lipid synthesis, secretion and storage in the liver has been investigated in male chickens in relation to the FA desaturation process. Seven birds fed on a low-lipid diet were administrated IM injections of 20 mg/kg of 17 β-estradiol in propylene glycol (four injections in 1 week), whereas four control birds received injections of propylene glycol only.