



## **Hepatic apo B and mRNA apo B levels in the underfed high-producing dairy cow during early lactation**

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maximal steatosis as early as week 2 (168 and 292 mg/10<sup>9</sup> cells, respectively). Our results showed that NEFA is the best plasma predictor for TG liver infiltration in weeks 1 ( $r = 0.68$ ), 2 ( $r = 0.42$ ) and 4 ( $r = 0.57$ ). Furthermore, plasma LDL, apo B and A-I are other potential predictors with the accuracy of prediction dependent on the time post-partum.

**Insulin uptake and effect on glucose utilization by ovine and bovine adipose tissue cultured over 7 days.** Y Faulconnier, L Guillon, R Lefaivre, M Tourret, Y Chilliard (*INRA, Laboratoire Sous-Nutrition des Ruminants, 63122 Saint-Genès-Champanelle, France*)

The effect of insulin (2 mU/ml) on glucose utilization was studied on adipose tissue (AT) explants from non-lactating non-pregnant cows ( $n = 5$ ) and ewes ( $n = 5$ ) fed a restricted diet (20–22% of energy maintenance requirement, EMR) for 8–10 d and then overfed (188 or 228% of EMR, for cows or ewes) during 21 (cows) or 10 (ewes) d until slaughter, to induce a rebound in lipogenic activities. The body condition (scale 0–5) of the cows and ewes averaged  $2.5 \pm 0.6$  and  $3.0 \pm 0.3$ , and the mean adipocyte diameter  $122 \pm 3$  and  $111 \pm 9$   $\mu\text{m}$ , respectively.

Samples of perirenal AT were cut into 10–15 mg pieces, and cultured over 7 d in sterile conditions in medium 199 supplemented with acetate (7.0 mM). The culture medium was changed daily. Glucose concentration was measured using the glucose dehydrogenase method. The loss of insulin from the cultured medium in the 2 species ranged from 60 (1st d of culture) to approximately 35% (d 2 to d 7) of the initial amount.

In basal conditions, the glucose utilization was similar (33–24 and 16  $\mu\text{mol}/24 \text{ h}/10^6$  adipocytes on d 1–2, 3–4 and 5–6–7, respectively) in the 2 species. However, the glucose utilization by bovine AT was higher (+18%) during the 2nd than during the 1st d, in contrast to ovine AT where this utilization decreased (–13%) during this period. In the 2 species, the glucose utilization then progressively decreased until d 7.

The addition of insulin increased ( $P < 0.001$ ) glucose utilization in the 2 species. However, the effect of insulin was different according to the species and the day of culture (interaction species  $\times$  insulin  $\times$  day,  $P < 0.03$ ). The effect of insulin on

d 1 was greater in bovine (+67%) than in ovine (+20%) AT, whereas on d 3–4 and d 5–6–7 this effect was greater in ovine (+92 and 132%) than in bovine (+64 and 50%) AT.

This study indicates that ruminant AT explants remain metabolically active for at least 7 d when maintained in a suitable medium, with interactions between insulin and animal species that affect glucose utilization.

**Hepatic apo B and mRNA apo B levels in the underfed high-producing dairy cow during early lactation.** D Gruffat<sup>1</sup>, F Duboisset<sup>1</sup>, D Durand<sup>1</sup>, J Lefaivre<sup>1</sup>, A Ollier<sup>2</sup>, G d'Onofrio<sup>3</sup>, P Williams<sup>4</sup>, Y Chilliard<sup>2</sup>, D Bauchart<sup>1</sup> (<sup>1</sup> *INRA, Laboratoire Croissance et Métabolismes des Herbivores*; <sup>2</sup> *INRA, Laboratoire Sous-Nutrition des Ruminants, 63122 Saint-Genès-Champanelle*; <sup>3</sup> *INSERM U 321, Hôpital de la Pitié, 75013 Paris*; <sup>4</sup> *Rhône Poulenc Nutrition Animale, 03600 Commentry, France*)

In high-producing dairy cows in early lactation, intense mobilization of lipids is often associated with fatty liver syndrome. The limited capacity of the liver to export triglyceride-rich lipoproteins in the form of very low density lipoproteins (VLDL) increases the risk of development of fatty liver. In cows in early lactation, synthesis of apolipoprotein B (apo B), the major apolipoprotein in VLDL, may be a rate-limiting step for hepatic VLDL production and secretion.

Eight multiparous H  $\times$  F fat cows after calving (body condition score: 3.9/5) were offered a low concentrate (< 25% DM diet) – corn silage complete diet to increase fat mobilization and to induce hepatic steatosis. All cows were liver biopsied (400 mg/cow; samples frozen in liquid nitrogen) at 1, 2, 4, and 12 weeks after calving. Total hepatic RNA was extracted from each biopsy sample using guanidium thiocyanate/phenol/chloroform. Levels of mRNA for apo B were determined by the Dot-blot method using a cDNA human probe and hepatic apo B levels were measured by Western-blot using rabbit specific antibody against bovine apo B. Hepatic DNA was determined by fluorimetric method and was used to express the apo B levels per 10<sup>9</sup> hepatic cells.

Levels of mRNA of apo B remained stable after calving ( $8.4 \pm 1.0$ ,  $8.3 \pm 1.8$ ,  $10.6 \pm 1.3$ , and

11.3  $\pm$  1.2 arbitrary units (AU) / $\mu$ g of total RNA ( $\pm$  SE) for weeks 1, 2, 4, and 12, respectively). Similarly, levels of hepatic apo B were unchanged between weeks 1 and 4 (2.5  $\pm$  0.3, 3.1  $\pm$  0.5, 3.5  $\pm$  0.5 AU/10<sup>9</sup> cells ( $\pm$  SE) for weeks 1, 2, and 4, respectively). However, in week 12, apo B levels were 3.7 times higher (9.4  $\pm$  2.4 AU / 10<sup>9</sup> cells,  $\pm$  SE,  $p < 0.01$ ) than in weeks 1–4.

These results indicate no changes in the level of hepatic apo B gene expression during the first 4 weeks of lactation although lipid infiltration increases by 85% during this period. However, the dramatic accumulation of hepatic apo B recorded in week 12, which occurred without changes in levels of mRNA, may have resulted from either a higher rate of tranlaction and/or a decrease in the rate of intracellular apo B degradation.

**Effects of dietary cholesterol and fatty acids on the lipid composition of muscle in the preruminant calf.** L Leplaix-Charlat, D Durand, C Legay, C Leoty, R Souchet, D Bauchart (*INRA, Laboratoire Croissance et Métabolismes des Herbivores, 63122 Saint-Genès-Champanelle, France*)

Muscle lipids determine the dietetic and organoleptic qualities of meat in various species. In the preruminant calf, a functional monogastric, lipid composition of muscle tissues can be modulated by nutritional factors, mainly by the lipids of the milk replacer. The combined effects of the ratio of saturated to polyunsaturated (PUFA) fatty acids and the cholesterol in the milk replacer on lipid composition of the rectus abdominis (RA) muscle in the preruminant calf have been determined.

Twenty-two 4-week-old male F x H calves (65  $\pm$  5 kg BW) were fed for 17 d one of the following diets: 1) a conventional milk replacer (5.2 Mcal/kg DM) containing triglycerides (TG, 23% diet DM) from either tallow (T, 4% of PUFA n-6;  $n = 6$ ) or soyabean oil (S, 57% of PUFA n-6;  $n = 5$ ); or 2) the same diets (T and S) supplemented with cholesterol (1% diet DM) (TC,  $n = 6$ ; SC,  $n = 5$ ).

A muscle (RA) sample (6 g) was taken at slaughter and immediately frozen in liquid nitrogen. Total lipids were extracted in chloroform/methanol (2:1, V/V) and TG, total cholesterol (TC) and phospholipids (PL) were determined by enzymatic or colorimetric methods. TG and PL were also isolated by thin-layer

chromatography and their fatty-acid (FA) composition was determined by gas-liquid chromatography.

Substitution of tallow for soyabean oil in the milk diet did not modify significantly mean liveweight gain (LWG; 0.9 kg/d). It increased total lipids in RA muscle (11.1 vs 8.6 mg/g of fresh tissue), because of the 2.2 times increase of TG ( $P < 0.05$ ). Similar variations were observed with the TC diet compared to the T diet (11.5 vs 8.6 mg/g). On the other hand, addition of cholesterol to the S diet led to a decrease of total lipids (8.9 vs 11.1 mg/g,  $P < 0.05$ ), mainly because of a decrease in TG (-38%). Compared to the diet T, the PUFA-rich diet increased the C18:2 n-6 proportion in TG (x 16;  $P < 0.05$ ) and PL (x 2;  $P < 0.05$ ) to the detriment of C18:1 n-9. Addition of cholesterol to diets T and S did not modify the LWG or FA composition of total lipids in RA muscle.

Dietary lipids only affect the TG fraction of lipids in RA muscle. Dietary PUFA n-6 favour TG synthesis by muscle tissue, probably because of the stimulation of TG-rich lipoprotein production by the liver, as described elsewhere in the calf. Finally, addition of cholesterol does not lead to a higher cholesterol content in RA muscle.

**Étude de la variation de composition en acides gras des tissus adipeux de petits ruminants : analyse par GC/MS.** A Rouzeau <sup>1</sup>, P Bas <sup>1</sup>, L Eveleigh <sup>2</sup>, D Sauvart <sup>1</sup> (<sup>1</sup> Laboratoire associé de nutrition et alimentation INRA de l'INA PG; <sup>2</sup> Laboratoire de chimie analytique de l'INA-PG, 16, rue Claude-Bernard, 75231 Paris Cedex 05, France)

Les tissus adipeux sous-cutanés des petits ruminants ont la particularité de pouvoir s'enrichir en acides gras ramifiés (AGR). La spectrométrie de masse est utilisée pour déterminer la structure des acides gras (AG) et tenter d'en déduire leur origine (micro-organismes du rumen ou synthèse endogène).

Quatre tissus adipeux (2 internes : omental et périséal, et 2 sous-cutanés : sternal et caudal) sont étudiés chez 2 agneaux et 2 chevreaux abattus vers 25 kg de poids vif (14 et 17 sem d'âge, respectivement). Les animaux sont alimentés depuis l'âge de 7 sem avec une ration complète