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Protein synthesis in the liver is differently altered by the dietary supply of nitrogen/energy ratio in lambs.

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Introduction

The liver is one of the major competitors of muscles for amino acid (AA) utilization within the body. Indeed, its important net AA uptake (45% of net portal appearance) and intense protein turnover competes with the peripheral tissues for the utilisation of AA (Lapierre et al., 2005, Kraft et al., 2008). The liver is also at the crossroad of various metabolic pathways involving AA and energetic nutrients (protein synthesis, gluconeogenesis, ureagenesis, and oxidation) (Lapierre et al, 2005). The distribution of AA between the different pathways must be tightly regulated to respond to nutritional challenges and fit with the demand of peripheral tissues in nutrients. The supply of nutrients to the liver is partly responsible for the regulation of each of the pathways utilizing those nutrients.

The aim of this study was to measure the liver protein synthesis adaptability to a low energy (E) or low nitrogen (N) feed supply compared to a well balanced diet (C). Two questions were addressed: first, to quantify the impact of dietary imbalances on hepatic

protein synthesis, and second, to dissociate in the response what is due to a mass action law of nutrient supply from metabolic adaptations of hepatic tissues. Therefore, two complementary approaches were used with animals fed the same three diets (C, N and E). A first experiment was conducted *in vivo* with sheep being catheterized at the splanchnic level. A second experiment was conducted *ex vivo* with liver slices to study the specific response of the liver. In the *ex vivo* model of liver slices, interactions between tissues and organs do not occur and it was chosen to maintain hormone and nutrient concentrations in the medium at similar levels for all treatments in order to evaluate the influence of the experimental diets on the metabolic adaptations of the liver. The comparison of results between the two approaches will highlight some of the regulatory mechanisms of hepatic protein synthesis following a low nitrogen or low energy supply in the diet.

Materials and Methods

Three diets were tested, composed of 70% concentrate and 30% hay : 1) a control (C), well balanced diet for nitrogen and energy that allowed a theorical growth of 200g a day according to INRA (Jarrige, 1989), 2) a diet allowing a 20 % lower nitrogen relatively to metabolizable energy supply (N), and 3) a diet allowing a 20% lower metabolisable energy relatively to nitrogen supply (E). Corn and its components (corn starch and corn gluten) were the main ingredients of the concentrates. Their proportions varied between the three diets in order to induce lower nitrogen or energy contents. Diet digestibility was measured in both experiments.

In the *in vivo* experiment, 6 lambs (41.5 ± 2.6kg) were used in a Latin Square design. The animals were catheterized at the splanchnic level to measure net splanchnic AA and total volatile fatty acids (C2 + C3 + C4) uptake by the liver as well as insulin concentrations in portal blood. A ^{13}C-Leucine infusion in the vena cava allowed the measurement of liver exported protein synthesis.

In the *ex vivo* experiment, 18 lambs (29.7 ± 0.5kg) were distributed into 6 blocks according to their initial body weights. In each block, the animals were fed one of the 3 experimental diets. At slaughter, liver slices were incubated *ex vivo* in a minimum physiological medium (see composition in Table 1) and ^{14}C Valine was added to measure exported protein synthesis.

Results

The weight (and age) of the animals differed between the two experiments, but the responses in term of intake, growth, and nitrogen balance to the three diets followed the same pattern of change (Kraft et al., 2008; Table2). Consequently, the results from both experiments were compared.

As expected from the experimental design (*in vivo* study) the AA supply to the liver via the portal vein was decreased with the N diet (-39%, $P = 0.005$) and the total volatile fatty acids supply was decreased with the E diet (-28%, $P = 0.01$). Insulin concentrations in the portal blood was only decreased in the E diet (-25%, $P = 0.04$ compared with C) (Table 3).

Exported hepatic protein synthesis was decreased with the E diet only (-12%, $P = 0.01$ compared to control), whereas it was similar between C and N diet ($P = 0.83$). Results obtained *ex vivo* were quite different to those obtained *in vivo*. Indeed, with N diet, the exported protein synthesis was increased compared with C (+46%, $P = 0.02$) whereas it remained unchanged with E diet ($P = 0.46$).

Discussion

With the N diet, the apparent discrepancy observed *ex vivo* and *in vivo* for exported protein synthesis rates suggests specific adaptations of the liver protein synthesis rate itself. *Ex vivo,* the efficiency of utilisation of AA by hepatocytes was increased with N. *In vivo,* this increased efficiency compensated the lower AA supply to the liver with the result that exported protein synthesis was sustained. An increased affinity (or activity) of some AA transport systems may be assumed as they are known to be stimulated during starvation (Fehlman et al.,1979).

Concerning the E diet, the absence of treatment effect *ex vivo* implies that the inhibition of exported protein synthesis observed *in vivo* may be due to changes in external regulatory factors (ie., nutrients or hormonal supply to the liver) or in the sensitivity/response of the tissue to hormones (which were not present in the medium *ex vivo*). The decreased portal supply of volatile fatty acids associated with decreased insulin concentrations (and probably an alteration of other hormones such as glucagon) may have down regulated hepatic protein synthesis *in vivo* (De Feo and Lucidi, 2002).

Conclusions

The combination of *in vivo* and *ex vivo* results *improv*ed the understanding of some regulatory mechanisms of hepatic exported protein synthesis. These mechanisms are very different depending on the nature of the dietary imbalance (energy or nitrogen). Indeed, when the nitrogen supply is decreased relatively to the energy supply (N diet), the efficiency for AA utilisation is increased to counteract the decreased supply of AA via the portal vein (increased AA transport?), which allowed to sustain hepatic protein synthesis. The AA used for protein synthesis are hence protected from further oxidation by the liver or other tissues and plasma proteins may represent a form of temporary storage (De Feo et al., 1992; Kraft et al, 2008). The mechanisms responsible for the decreased exported protein synthesis shown with the E diet (energy supply decreased relatively to nitrogen) are still to be determined and may involve nutrients and hormones supply to the liver which are connected to both intake and peripheral tissues utilization.

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Table 1: Medium composition (m*M*) used for the incubation of liver slices.
Salts: NaCl 120, NaHCO3 25; KCl 2.9; CaCL2 2.5; MgSO4 1.2; KHPO4 1.2,
Hepes: 5
BSA: 0.1 g/L
Amino acids: Ala 0.2, Asn 0.05, Asp 0.2, Cys 0.01, Cyst 0.075, Glu 0.12, Gln 0.03, Gly 0.5, His 0.1, Ile 0.1; Leu 0.15, Lys 0.125, Met 0.02, Phe 0.075, Pro 0.1; Ser 0.1; Thr 0.15; Trp 0.02; Tyr 0.15; Val 0.2
Propionate: 0.2
pH adjusted at 7.4

Table 2: Lambs live weight (kg), metabolisable energy intake (MJ/d), digested nitrogen (g/d) and ratio of metabolizable energy intake to digested nitrogen (MJ/g) for lambs fed a control (C), low nitrogen (N) or low energy (E) diet in : 1.the *in vivo* study on multicatheterized lambs; 2. the *ex vivo* study on liver slices.

	Diet				*P*
	C	N	E	SEM	value
Expt. 1 (in vivo, multi catheterized lambs)					
Average live weight (kg)	42.2[a]	41.7[a]	40.7[b]	0.30	0.02
Intake					
ME (MJ/d)	14.1[a]	14.2[a]	11.2[b]	0.14	<0.001
Digested Nitrogen (g/d)	16.2[a]	12.4[b]	14.7[a]	0.65	0.01
Digested Nitrogen/ME (g/MJ)	1.15[a]	0.87[b]	1.31[c]	0.013	<0.001
Expt. 2 (ex vivo, liver slices)					
Average live weight (kg)	30.0	29.7	28.9	0.45	0.20
Intake					
ME (MJ/d)	8.77[a]	8.69[b]	7.08[c]	0.022	<0.001
Digested Nitrogen (g/d)	9.51[a]	6.09[b]	10.10[a]	0.23	<0.001
Digested Nitrogen / ME (g/MJ)	1.08[a]	0.70[b]	1.42[c]	0.012	<0.001

Table 3 : Net portal appearance (mmol/h) of total amino acids (TAA) and total volatile fatty acids (VFA), and portal insulin concentration (nUI/L) for lambs fed a control (C) , low nitrogen (N) or low energy (E) diet in the *in vivo* study with multicatheterized lambs.

	Diet				*P* value
	C	N	E	SEM	
Net portal appearance (mmol/h)					
TAA	30.9 [a]	19.0 [b]	25.4 [a]	2.04	0.01
VFA	187.4 [a]	186.4 [a]	135.0 [b]	8.81	<0.01
Portal insulin concentration (nUI/L)	89.5 [a]	87.2 [a]	67.3 [b]	6.3	0.07

Figure 1: *In vivo* hepatic exported proteins synthesis measured in lambs (n = 6) fed a control (C), low nitrogen (N) or low energy (E) diets.

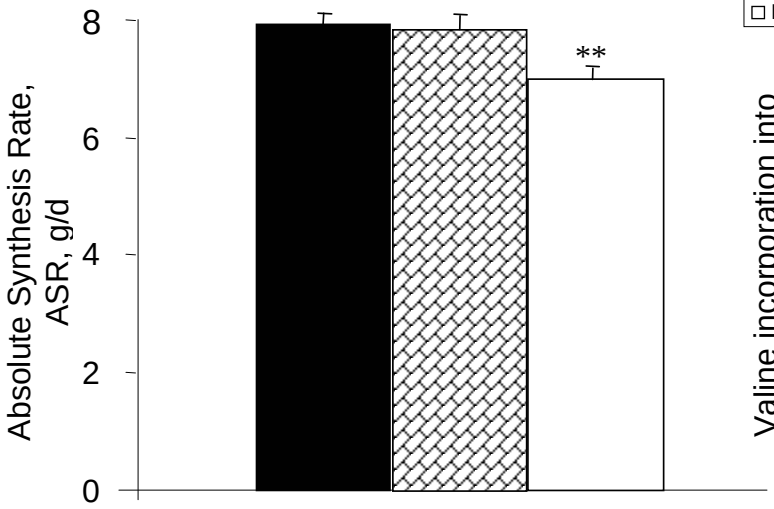
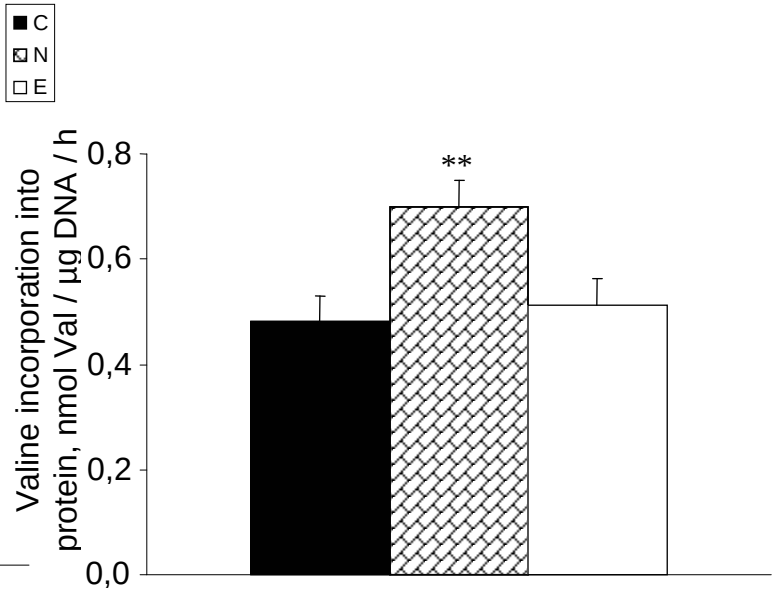


Figure 2. *Ex vivo* hepatic exported proteins synthesis measured in liver slices issued of lambs (n = 18) fed a control (C), low nitrogen (N) or low energy (E) diets.



** Means differ ($P < 0.05$) for diet effect.