

exceed the capacities of intestinal glutaminolysis, the dietary protein level has to be high. However, even with a large supply of protein, the splanchnic tissues continue to play a minor role in producing glutamine for muscles. In conclusion, under most usual nutritional conditions, there is an enterohepatic cycling of glutamine; the hepatic release of glutamine (and glutamate) may be interpreted as a nitrogen-salvaging process, independent of the acid/base status that becomes operative when the urea cycle activity is low.

Kinetic study of apoB-100 metabolism in non-insulin-dependent diabetics. C Maugeais, P Mahot, K Ouguerram, M Krempf, T Magot (*Centre de recherche en nutrition humaine, hôpital Laënnec, 44035 Nantes cedex 01, France*)

Non-insulin-dependent diabetic (NIDDM) subjects exhibit abnormalities in their plasma lipid and lipoprotein profiles that increased their risk of cardiovascular diseases. This study was designed to examine the metabolic behavior of apoB-100 using stable isotopes.

The five patients (37–66 years old) with NIDDM (HbA1C 6.7–10.3%) had hypertriglyceridaemia (TG 2.9 ± 0.9 g/L). They received a constant infusion (10^{-6} mol/kg/h) of deuterated leucine during 14 h. Patients were in fasting state during the experiment. Lipoproteins were isolated by ultracentrifugation and apolipoproteins by electrophoresis (SDS-PAGE). ApoB-100 was hydrolyzed and the tracer-to-tracee ratio in leucine was determined by mass spectrometry. Kinetic analysis of the tracer-to-tracee curves was performed by multicompartimental modeling.

Compared to control subjects, very low density lipoprotein (VLDL)-apoB production was higher (41.5 vs 24.8 mg/kg/day). In the experimental diabetic subjects, fractional

catabolic rates (FCR) of VLDL-, and intermediate density lipoprotein (IDL)-apoB decreased (0.16 ± 0.05 vs 0.48 ± 0.05 h⁻¹, 0.15 ± 0.04 vs 0.62 ± 0.08 h⁻¹, respectively), while FCR of low density lipoprotein (LDL)-apoB slightly increased (0.030 ± 0.006 h⁻¹ vs 0.022 ± 0.002 h⁻¹).

The main perturbations of lipoprotein metabolism in NIDDM were upstream of LDL as an increase in VLDL synthesis and as a decrease in VLDL and IDL catabolism.

Isotopic enrichment kinetics of the nitrogenous milk fractions in goats receiving a single intravenous injection of L-(U-¹⁴C)-leucine. X Rubert-Aleman, G Rychen, C Claudon, F Laurent (*Ensaia-Inra, BP 172, 54505 Vandœuvre-les-Nancy, France*)

The aim of this experiment was to study the ¹⁴C-labelling kinetics of goat milk nitrogenous fractions (casein fraction [CN], whey protein [WP] and the nonprotein fraction [NPN]) after a single intravenous injection of L-(U-¹⁴C)-leucine, and to measure the apparition times of CN and WP labelling peaks to identify the labelled goat milk proteins which could be used for metabolic studies in monogastric animals (rat or pig).

L-(U-¹⁴C)-leucine (Amersham, UK) was used as an adapted marker for three main reasons: its metabolic pathways are well defined (transamination of leucine to α -ketoisocaproate [KIC], decarboxylation or reamination of KIC to leucine), leucine is particularly abundant in milk proteins and its extraction rate from the plasma to the mammary gland is very high [Mephram and Linzell (1966) *Biochem J* 101, 76-83].

Three lactating Alpine goats (3 L milk/day) were each fitted with two temporary intravenous catheters placed respectively in each jugular vein. Isotope injection of L-(U-¹⁴C)-leucine (3.6×10^6 Bq/goat diluted in 5 mL 0.9 % NaCl solution) was

carried out in the left catheter and the venous blood samples were taken in the right one. After the injection, the goats were milked out at 0.5, 1, 3, 5, 7, 9, 24, 32, 48, 56 and 72 h and blood samples were collected at 5, 10, 15, 20, 25, 30 min and at 1, 2, 3, 4, 5, 6, 7, 8, 9, 24, 32, 48, 56 and 72 h after the leucine injection.

In the deproteinized plasma, radioactivity levels quickly decreased (during the first 45 min) to a very low level. Labelled CN and WP were detected as early as half an hour after the injection. After 3 h, a labelling peak was observed in CN (126 ± 23 Bq/mg N), in WP (179 ± 32 Bq/mg N) and in the NPN fraction (117 ± 16 Bq/mg N). In the milk samples, the radioactivity of the different nitrogen fractions decreased exponentially until 72 h after ^{14}C -leucine injection (residual radioactivity level: 3.5 ± 1.3 Bq/mg N). The different apparition time of the peaks in plasma and milk proteins demonstrated the presence of intermediate storage pools for ^{14}C -leucine in the mammary gland [Bequette et al (1994) *Br J Nutr* 72, 211-220]. After 48 h, the radioactivity recovered in goat milk was 3.7×10^5 Bq/goat, or 10% of total radioactivity injected in the jugular vein.

This isotopic labelling study showed a high synthesis rate for CN and WP and indicated a feasible and adequate way to label goat milk proteins which could be used for metabolic studies in monogastric animals. This experiment completed previous experiments conducted by Mahé et al (1994) or Boirie et al (1995), who produced labelled cow milk proteins.

Postprandial splanchnic utilization of amino acids in elderly men. Y Boirie, P Gachon, E Verdier, L Morin, B Beaufrère (*Laboratoire de nutrition humaine, université Clermont-Auvergne, CRNH, Clermont-Ferrand, France*)

The major characteristic of ageing is a progressive loss of lean body mass (LBM), particularly of muscle proteins. This protein loss leads to decreased muscle strength and to unsuitable metabolic responses to stresses or trauma. It could result from changes in amino acid disposal between the muscle and splanchnic tissues during feeding, since a higher amino acid gut/liver uptake would limit amino acid availability for muscle. To test this hypothesis, postabsorptive (PA) and postprandial (PP) modifications of protein metabolism were assessed in six young men (YM: 22.6 ± 0.9 years, mean \pm SE) and six old men (OM: 68.2 ± 1.9 years) using a combination of labelled oral ($^2\text{H}_3$ -leucine) and intravenous leucine (^{13}C -leucine) to determine the splanchnic extraction of leucine and other whole body components of protein metabolism. The test meal was identical for both groups: 10 Kcal/kg, 16% protein, administered in small fractions every 15 min for 4 h. Body composition was measured by bioelectrical impedance analysis.

Leucine flux and oxidation were higher in the YM, either during the PA state (flux = 1.54 ± 0.09 vs 1.25 ± 0.07 , Ox = 0.41 ± 0.02 vs 0.30 ± 0.04 $\mu\text{mol/kg/min}$, YM vs OM, $P < 0.05$) or in the fed state (flux = 1.99 ± 0.09 vs 1.59 ± 0.15 , Ox = 1.01 ± 0.04 vs 0.68 ± 0.08 , YM vs OM, $P < 0.05$). When expressed per unit LBM, PA-, PP leucine flux and PA oxidation rates were not significantly different in the two groups, but PP oxidation remained lower in the elderly men whatever the mode of expression (0.96 ± 0.08 vs 1.18 ± 0.06 $\mu\text{mol/kg LBM/min}$, OM vs YM, $P < 0.05$). Splanchnic extraction of leucine was much higher in the elderly (50 ± 11 vs $22 \pm 2\%$, $P < 0.05$), and was inversely related to PP oxidation ($r = -0.93$, $P < 0.001$) and plasma leucine concentrations ($r = 0.71$, $P < 0.01$).

In conclusion, in both postabsorptive and fed states, leucine fluxes per unit body weight were lower in elderly men, but the