conditions studied so far [Attaix *et al* (1994) *op cit*], and could presumably be responsible for the breakdown of myofibrillar proteins.

Coordinate activation of lysosomal, Ca^{2+}- and ATP-ubiquitin-dependent proteolytic pathways in the soleus muscle in hind-limb suspended rats. D Taillandier [1], E Aurousseau [1], A Obled [1], D Béchet [1], M Ferrara [1], P Cottin [2], A Ducastaing [2], X Bigard [3], CY Guézennec [3], D Attaix [1] (*[1]INRA-Theix, Centre de Recherche en Nutrition Humaine et Unité d'Étude du Métabolisme Azoté, 63122 Saint-Genès-Champanelle; [2] ISTAB et Université de Bordeaux I, 33405 Talence; [3] Centre d'Études et de Recherches de Médecine Aérospatiale, 91228 Brétigny-sur Orge, France*)

During simulated weightlessness, increased protein breakdown is the major determinant of muscle atrophy and protein wasting in antigravity muscles, *ie* the soleus [Thomason and Booth (1990) *J Appl Physiol* 68, 1-12]. The aim of this study was to identify the proteolytic pathways responsible for these alterations in skeletal muscle.

Twenty male Wistar rats with an initial body weight of 120 ± 5 g were randomly assigned to a control and a non-weight-bearing group. The experimental group was suspended by the tail according to the protocol described by Taillandier [(1993) Thèse de Doctorat de l'Université Blaise-Pascal, Clermont-Ferrand II, France]. Animals were studied after 9 d of treatment. Isolated soleus muscles were incubated to measure protein synthesis and breakdown simultaneously, as described by Tischler *et al* [(1982) *J Biol Chem* 257, 1613-1621]. Two other groups of rats raised in identical conditions were used to measure either the rate of proteolysis after inhibiting both lysosomal and Ca^{2+}-dependent proteases [Wing and Goldberg (1993) *Am J Physiol* 264, E668-E676], or mRNA levels for multiple components of proteolytic systems, in the soleus muscle.

The soleus muscle atrophy was higher than 50% ($P < 0.001$) after 9 d treatment. Total protein breakdown of suspended rats was enhanced by 66% ($P < 0.001$), compared to control animals. By contrast, the rate of protein synthesis was not significantly modified by hindlimb suspension. The lysosomal and Ca^{2+}-dependent proteolysis was greatly enhanced (254%, $P < 0.001$) in the soleus muscle of non-weight-bearing rats, but did not exceed 18% of total proteolysis. In addition, the inhibition of both lysosomal and Ca^{2+}-dependent proteases did not suppress increased proteolysis in the soleus muscle of suspended rats. Northern blot procedures revealed large increases in mRNA levels for lysosomal (cathepsins B, L and D), and Ca^{2+}-dependent (m-calpain) proteases, and for components of the ATP-ubiquitin-dependent proteolytic pathway (ubiquitin, 14-kDa ubiquitin carrier protein E2, C2 and C9 proteasome subunits) in the soleus muscle of non-weight-bearing rats. Similar data were observed in a phasic muscle, the extensor digitorum longus.

These data clearly demonstrate that the major proteolytic systems are coordinately activated in skeletal muscle during simulated weightlessness. Cathepsins, calpains and the proteasome could degrade different classes of proteins, the ATP-ubiquitin-dependent proteolytic pathway being presumably responsible for the breakdown of the major contractile proteins.

VII. Nutrition and pathology

Effects of moderate amounts of dietary fat on post-prandial lipemia in healthy human subjects. C Dubois [1], M Armand [1], P Borel [1], M Senft [1], H Portugal [2], AM Pauli [2], PM Bernard [2], V Azais-Braesco [3], C Latge [4], D Lairon [1] (*[1] Unité 130-INSERM (National Institute of Health and Medical Research), 18, avenue Mozart, 13009 Marseille; [2] Hôpital Sainte-Marguerite, 13000 Marseille; [3] Unité Vitamines, LNSA-INRA, 78000 Jouy-en Josas; [4] Crealis, Brive, France*)

There have been considerable developments in postprandial studies on human subjects during recent years. These experiments have generally studied blood postprandial responses to amounts of fat (70–140 g) greatly exceeding that usually ingested during a meal, even for a western high-fat diet (40–50 g per meal).

The present study therefore compares the post-prandial responses to 2 different and moderate amounts of dietary fat (30 and 40 g) in the form of well-defined dietary emulsions of sunflower oil (droplet size: 30–44 μm). The 30 g fat

test meal corresponded to a meal in accordance with dietary guidelines (30–35% total energy provided by fat), and the 40 g test meal was closer to the western diet.

Eight normolipemic males ingested on separate days and in a random order a full meal containing 4 slices French bread, 60 g wheat semolina (cooked and hydrated with 120 ml water), 2 cooked egg whites, 1 fat-free yogurt, a cup of coffee and the tested fat emulsion. The 30 g fat test meal provided 3 240 kJ (775 kcal with 13.3% proteins, 50.7% carbohydrates and 35.9% fat), and the 40 g fat test meal provided 3 649 kJ (873 kcal with 11.8% proteins, 44.8% carbohydrates and 43.4% fat). The amount of phospholipids was less than 0.2 g per meal and no cholesterol was present in the test-meals.

Blood samples were obtained in the fasting condition and after the meal intake *via* an iv catheter every hour for 7 h. Lipoproteins (chylomicrons, VLDL + chylomicrons remnants, LDL and HDL) were isolated by ultracentrifugation. Lipid parameters (triglycerides, free and esterified cholesterol, phospholipids) were determined on serum and on each class of lipoproteins using enzymatic procedures. Insulin was determined by an immuno-enzymatic method and apoproteins (A1, B) were determined by laser-nephelometry on serum.

The serum and chylomicron triglyceride response was strictly proportional to the amount of fat ingested and peaked after 2–3 h. After the 30 g fat test-meal, there were no significant changes over baseline in phospholipid, free and esterified cholesterol concentrations in serum for 7 h. Inversely, after the 40 g fat test-meal, serum phospholipids and free cholesterol significantly increased and esterified cholesterol decreased postprandially. At the same time, significantly different responses were observed after both meals for LDL-free cholesterol, VLDL and LDL esterified cholesterol and HDL phospholipids: the variation of the range was higher with the 40 g fat meal as compared to the 30 g fat meal. Insulin, ApoA1 and ApoB responses were comparable with the 2 test-meals.

The present data show that limited changes in fat intake (30 instead of 40 g fat) markedly affects postprandial lipemia and lipoprotein responses in normolipidic human subject. This might be taken into account for planning future postprandial studies, and suggests that postprandial lipid data may be useful tools for setting dietary guidelines.

Selective storage and mobilization of individual fish oil n–3 polyunsaturated fatty acids in adipose tissue. T Raclot, E Mioskowski, R Groscolas (*Centre d'Écologie et Physiologie Énergétiques, CNRS, 23, rue Becquerel, 67087 Strasbourg, France)*

The post-intake bioavailability, and thus the biological effects, of dietary n–3 polyunsaturated fatty acids (n–3 PUFAs) may depend on their storage in and mobilization from adipose tissue triglycerides (TG). Both aspects of the metabolism of the 4 major n–3 PUFAs (20:5n–3, 22:6n–3, 22:5n–3 and 18:4n–3) were studied in rats fed a high-fat (20%) fish oil (40% n–3 PUFAs) diet or a control diet (4% fat; 2% n–3 PUFAs in fat) for 4 weeks. According to the n–3 PUFA, 13–32% of its ingested mass was stored in fat reserves at the end of fish oil feeding. However, the storage was selective. The *in vivo* relative incorporation (% in TG / % in diet) increased significantly according to: 20:5n–3 (0.25) < 18:4n–3 (0.37) < 22:6n–3 (0.49) < 22:5n–3 (0.78). Thus, 20:5n–3 was 3-fold less incorporated than 22:5n–3. The *in vitro* relative mobilization of n–3 PUFAs (% in released FFA / % in TG) was determined by incubating pieces of adipose tissue under conditions of stimulated lipolysis (norepinephrine 10^{-6} M; bovine albumin 4% by weight in the medium). Relative mobilization was also selective, decreasing significantly according to 20:5n–3 (2.88) > 18:4n–3 (1.51) > 22:6n–3 (1.08) > 22:5n–3 (0.91). Thus, 20:5n–3 was almost 3 fold more mobilized than total fatty acids, including 22:5n–3 and 22:6n–3. This confirms previous results obtained by incubating isolated fat cells [Raclot and Groscolas (1993) *J Lipid Res* 34, 1515-1526]. The *in vivo* relative incorporation into adipose tissue was inversely and significantly related to the *in vitro* relative mobilization. The same results were obtained from rats fed the control diet, and from retroperitoneal and subcutaneous adipose tissues.

In conclusion, n–3 PUFAs are efficiently but selectively stored in adipose tissues of growing rats, whatever their dietary intake. The selectivity of the mobilization of individual n–3 PUFAs could contribute to their differential storage. The most preferential mobilization of 20:5n–3 could contribute to its maintenance in the circulation during or shortly after its supplementation, which could substain its various biological effects. On the other hand, the long-term storage of this fatty acid in fat stores is probably limited.