(DMEM/BSA). The amplitude of IGF-1 stimulated AIB uptake (about 300% of basal) and protein synthesis (about 130% of basal) did not differ between lines, whereas the IGF-1-stimulated DG uptake was higher in myotubes from the FG line than from the SG line ($F_{(1.69)} = 4, P < 0.01$; 168 ± 12% *vs* 145 ± 8% of basal, respectively). Following 24 h incubation of the myotubes in DMEM containing 0.5% Ultroser, protein degradation was similar in both lines (about 22%).

Our data show that DNA synthesis by satellite cells from FG chicks is higher than that of cells from SG chicks in the presence of FCS or IGF-1. On the contrary, metabolic parameters of satellite cell derived myotubes and their response to IGF-1 were not affected by selection except for glucose uptake. Therefore, some growth-related pathways, but not all, have been modified by genetic selection for faster growth.

Proteolytic pathways involved in muscular dystrophy in mdx, dy/dy and mdf mice: a preliminary study. L Combaret [1], D Taillandier [1], E Aurousseau [1], L Voisin [1], D Meynial-Denis [1], O Boespflug-Tanguy [2], JL Guénet [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] *Laboratoire de Biochimie, Faculté de Médecine, 63001 Clermont-Ferrand Cedex;* [3] *Unité de Génétique des Mammifères, Institut Pasteur, 75015 Paris, France*)

Alterations in the structural, functional and metabolic properties of skeletal muscle or protein loss observed in muscular dystrophy may result from variations in protein synthesis and/or protein breakdown. The aim of the present study was to identify the proteolytic systems responsible for such alterations using Northern blot procedures, since mRNA levels proteases could be a sensitive index of increased protein breakdown [Attaix *et al* (1994) *Reprod Nutr Dev* 583-597] in mdx (lacking dystrophin and reproducing the Duchenne de Boulogne myopathy) in dy/dy (exhibiting muscle atrophy presumably due to neuronal defects) and mdf mice (characterized by a preferential loss of type I fibers).

Three groups of mdx, dy/dy and mdf dysotrophic mice ($n = 3 - 6$) were studied at 6, 6 and 8 weeks of age respectively. mdx, dy/dy and mdf animals were compared to control Black 6, OF1, and Black 6 mice, respectively, of the same age. Muscle atrophy was estimated by comparing the tibialis anterior muscle mass divided by the body weight, to compare dystrophic and control mice with slightly different live weights. The Northern blot procedures used to measure mRNA levels for cathepsin D, m-calpain, ubiquitin, and proteasome subunits were described by Taillandier [(1993) Thèse de Doctorat de l'Université Blaise-Pascal, Clermont-Ferrand II, France].

The mdx mice did not exhibit any muscle atrophy. Only the mRNA levels for m-calpain, but not for cathepsin D or ubiquitin, were higher in mdx mice than in controls, suggesting a selective activation of the Ca^{2+}-dependent proteolytic pathway, in accordance with previous observations by Turner *et al* [(1988) *Nature* 335, 735-738]. By contrast, in dy/dy mice that present a small atrophy of the tibialis anterior muscle (26%, $P > 0.05$) only the expression of polyubiquitin was higher than in control mice, mRNA levels for cathepsin D, m-calpain and the C2 proteasome subunit being systematically lower than in controls. These data suggest a possible inhibition of the different proteolytic pathways in dy/dy mice. Finally, the large atrophy of the tibialis anterior muscle observed in mdf mice (71%, $P < 0.001$) probably totally resulted from impaired protein synthesis, since no variation in mRNA levels for cathepsin D, m-calpain, ubiquitin and C9 the proteasome subunit was observed in this model of dystrophy.

Taken together, these preliminary data clearly indicate that the activation of a given proteolytic system totally depends on the type of muscular dystrophy.

Effect of lipoprotein lipase polymorphisms on lipid levels in obese patients before and after a hypocaloric diet. R Jemaa, S Tuzet, F Fumeron, D Betoulle, M Apfelbaum (*INSERM, U 286, Faculté Xavier-Bichat, rue H-Huchard, 75018 Paris, France*)

Obesity is frequently associated with hypertriglyceridemia and hypoHDLemia [Criqui *et al* (1986) *Circulation* 73 (suppl 1), 140-150]. A hypocaloric diet is accompanied by a decrease in lipid levels. Individual variations in the response to dietary modification are likely to be attributable to both environmental and genetic factors. The lipoprotein lipase (LPL) plays a key role in the

catabolism of triglyceride rich lipoprotein. Several studies have reported associations between serum lipid (TG, HDL) levels and restriction fragment length polymorphisms (RFLPs) of the LPL gene (Pvu II and Hind III).

The aim of the present study was to assess the effect of genetic variations at the lipoprotein lipase (LPL) gene locus on lipid-related variables in obese subjects, before and after a hypocaloric diet.

Unrelated obese subjects (80 women and 40 men) were recruited on the basis of 120% of ideal weight as determined by Metropolitan Life Insurance tables of 1959. The mean (± SD) age of the subjects was 42 ± 10 years and the mean (± SD) body mass index (BMI) was 33.2 ± 5.4 kg/m^2. They were submitted to a restrictive diet (1 824 ± 311 kcal/d), containing 42% of energy as fat, 19.4% as protein and 37% as carbohydrates, during 3 months. Diabetic patients or patients using drugs known to modify lipid levels were excluded. All subjects were French Caucasians.

Two restriction length polymorphisms (RFLPs) (Pvu II and Hind III) were determined by enzymatic digestion of DNA after PCR amplification. Digestion with Hind III generated 2 alleles, H1 and H2, with frequencies of 0.28 and 0.72, respectively. Digestion with Pvu II generated 2 alleles, P1 and P2, with frequencies of 0.49 and 0.51, respectively.

On spontaneous diet, subjects with H2H2 genotypes had significantly higher TG in serum and VLDL ($P < 0.005$) than H1 subjects. After a hypocaloric diet, subjects with H2H2 genotypes reduced their total and VLDL-TG more than subjects with H1 allele.

According to Pvu II genotypes, there was no difference between lipid-related variables in obese subjects, either on a spontaneous diet or after the hypocaloric diet.

In conclusion, the lipoprotein profile is not the same for all obese people and depends on genetic factors, at least in part. The lipid response to hypocaloric diet also depends on genetic factors, which means that the benefits of such diets are not identical for all obese people.

VI. Protein metabolism

Increased ATP-ubiquitin-dependent proteolysis in skeletal muscles proximal to the tumor of Yoshida-sarcoma-bearing rats.

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Skeletal muscle protein wasting is commonly observed in cancer patients and tumor-bearing animals, and results from impaired protein synthesis and/or enhanced proteolysis [Pisters and Brennan (1990) *Annu Rev Nutr* 10, 107-132]. Experiments were carried out to demonstrate whether there is proximal effect of a tumor on skeletal muscle proteolysis, and a coordinate activation of the 3 major proteolytic systems of skeletal muscle (*ie* lysosomal, Ca^{2+}- and ATP-ubiquitin-dependent) in rats bearing a small (< 0.5% body weight) and nonmetastasic tumor.

Young male Wistar rats with an initial body weight of 50 g were randomly divided into 2 groups of 7 control and tumor-bearing animals. The tumor group received an injection of 60 mg of sarcoma homogenate in 0.3 ml saline into the left vastus lateralis muscle. Animals were sacrificed 5 d after tumor implantation. The rates of total protein synthesis and breakdown were measured in incubated extensor digitorum longus (EDL) muscles as described by Tischler *et al* [(1982) *J Biol Chem* 257, 1613-1621]. Proteolysis was also measured in the presence of inhibitors of lysosomal and Ca^{2+}-dependent proteases [Wing and Goldberg (1993) *Am J Physiol* 264, E668-676]. Tibialis anterior (TA) muscles were also excised for measuring the expression of proteolytic systems, using Northern blot procedures.

The protein masses of both EDL and TA muscles of the tumor-bearing leg were significantly reduced 5 d after tumor implantation ($P < 0.01$) compared to the other (control) leg. The rate of total protein breakdown was 30–40% higher ($P < 0.01$) in the EDL muscle from the tumor-bearing leg, compared with the control leg, but protein synthesis was unchanged. Neither saline nor heat-inactivated tumor cells injections resulted in muscle atrophy or increased proteolysis in the EDL muscle 5 d after treatments. Incubating EDL