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## Effects of age and divergent selection for body weight on muscle protein turnover in chickens

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muscles from tumor-bearing rats in the presence of inhibitors of the lysosomal and the  $\text{Ca}^{2+}$ -dependent proteolytic pathways did not reduce differences between rates of proteolysis in EDL muscles from the tumor-bearing and the control legs. mRNA levels for cathepsin B were similar in the TA muscles of the tumor-bearing and control legs, but higher mRNA levels for m-calpain were observed in muscles of the tumor-bearing leg. By contrast, when EDL muscles from tumor-bearing rats were incubated under ATP-depleting conditions, proteolysis was almost identical in the tumor-bearing and control legs. In addition, mRNA levels for ubiquitin, 14 kDa ubiquitin carrier protein E2 and C8 or C9 proteasome subunits markedly increased in the TA muscle of the tumor-bearing leg compared with the control-limb. By contrast, all the parameters measured in the control-limb of tumor-bearing rats were identical to those observed in control rats.

This study suggests that the Yoshida sarcoma stimulates proximally protein breakdown in skeletal muscle at an early stage of tumor development. This activation presumably involves the ATP-ubiquitin-dependent proteolytic pathway, and possibly the  $\text{Ca}^{2+}$ -dependent proteinases.

**Effects of age and divergent selection for body weight on muscle protein turnover in chickens.** S Tesseraud, JC Cammas, AM Chagneau (*INRA, Station de*

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Rates of muscle protein turnover were determined at 2 and 4 weeks of age in 2 lines of chickens selected from the Bresse-pile strain for high or low growth rate (HG and LG) by Ricard [(1975) *Ann Genet Sel Anim* 7, 427-443]. Protein fractional synthesis rates (Ks) in the Pectoralis major muscle were measured *in vivo* according to the flooding dose method [Garlick *et al* (1980) *Biochem J* 192, 719-723]. Ten min before slaughter, the 3-h-fasted animals received a single intravenous injection of unlabelled L-phenylalanine (150  $\mu\text{mol}/100\text{ g}$ ) combined with 1.85 MBq/100 g of L-[4- $^3\text{H}$ ] phenylalanine (Amersham, 0.96 TBq/mmol) to flood the precursor pools. Rates of protein breakdown (Kd) were estimated as the difference between the synthesis rates and the growth rates of muscle protein.

In 4-week-old chickens the fractional rates of protein synthesis were 60% of their values at 2 weeks of age for both lines (table I). This developmental decline, also observed in whole body for growing chickens [Muramatsu and Okumura (1985) *J Nutr* 115, 483-490], was related to decreased capacities for protein synthesis (Cs), *ie* RNA/protein ratio, whereas the ribosomal activity, *ie* the RNA activity or the translational efficiency kRNA, was constant (2 weeks:  $11.9 \pm 0.5$  and  $12.3 \pm 0.6\text{ mg}/(\text{mg}\cdot\text{d})$  in HG and LG lines respectively; 4 weeks:  $11.6 \pm 0.8$  and  $12.1 \pm 0.8\text{ mg}/(\text{mg}\cdot\text{d})$  respectively).

**Table I.** Effects of divergent selection for body weight on protein turnover in the pectoralis major muscle of 2 and 4-wk-old chickens (S Tesseraud *et al*).

Age (weeks)	Line <sup>1</sup>	n	Fractional rates (%/day)		Cs <sup>2</sup> ( $\mu\text{g}/\text{mg}$ )	Absolute amounts (mg/d)	
			Ks	Kd		Synthesis	Degradation
2	HG	10	16.6 <sup>a</sup>	2.1 <sup>a</sup>	13.9 <sup>a</sup>	157.2 <sup>a</sup>	22.2 <sup>a</sup>
	LG	7	18.8 <sup>a</sup>	4.3 <sup>b</sup>	15.2 <sup>a</sup>	71.2 <sup>b</sup>	16.8 <sup>a</sup>
4	HG	10	10.6 <sup>b</sup>	5.8 <sup>b</sup>	9.2 <sup>b</sup>	278.3 <sup>c</sup>	152.8 <sup>b</sup>
	LG	11	11.1 <sup>b</sup>	4.3 <sup>b</sup>	9.1 <sup>b</sup>	117.3 <sup>d</sup>	46.2 <sup>a</sup>
	Pooled SE		0.9	0.7	0.4	10.3	9.6

Means for 7–11 chickens. Values not sharing a common subscript were significantly different ( $p < 0.05$ ). <sup>1</sup> Lines of chickens selected for high or low growth rates (HG and LG). <sup>2</sup> Ribosomal capacity.

At 2 weeks of age, protein fractional breakdown rates were significantly higher in line LG compared to line HG, showing divergence in the rate of muscle protein degradation with selection for growth. By contrast, the usual major determinant of accretion, protein fractional synthesis rates remained unchanged, as previously suggested by Tomas *et al* [(1991) *Br Poult Sci* 32, 363-376] in chickens. In agreement with this, Seve *et al* [(1990). *In: Proc 4th Felasa Symposium* (10-15 June 1990, Lyon) 69-73] also recorded in 2 extreme genotypes of pigs an higher muscle protein turnover in the LG than in the HG line.

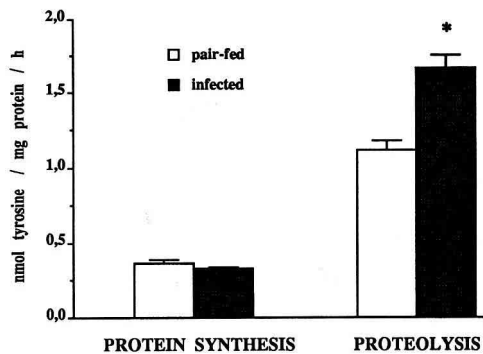
In 4-week-old chickens the difference between protein fractional breakdown rates between the lines was abolished, suggesting that changes in protein metabolism occur principally in the first days of life. However, when expressed in terms of absolute amount, protein deposition (g/d) was still higher in the fast growing line (2.5 or 1.8 times higher at 2 or 4 weeks of age).

### Involvement of Ca<sup>2+</sup>- and ATP-ubiquitin-dependent proteases in increased skeletal muscle proteolysis in septic rats.

L Voisin<sup>1</sup>, D Taillandier<sup>1</sup>, E Arousseau<sup>1</sup>, L Combaret<sup>1</sup>, D Breuillé<sup>2</sup>, C Obléd<sup>1</sup>, D Attaix<sup>1</sup> (<sup>1</sup>*INRA-Theix, Centre de Recherche en Nutrition Humaine et Unité d'Étude du Métabolisme Azoté, 63122 Saint-Genès-Champagnelle;* <sup>2</sup>*Clintec Technologies, 78148 Vélizy, France*)

In bacterial infection, muscle protein wasting is partly due to anorexia. The aim of the present studies was to measure variations in both protein synthesis and breakdown in incubated muscles, and to identify the proteolytic pathways activated in infected rats.

Two groups of 8 rats (approximate initial body weight 300 g) were intravenously injected with 6 x 10<sup>8</sup> live *E coli* or saline. Control animals were pair-fed with infected rats. Animals were studied 2 d post-injection. Protein synthesis and breakdown measured *in vitro* using isolated epitrochlearis muscles, as described by Tischler *et al* [(1982) *J Biol Chem* 257, 1613-1621]. mRNA levels for proteases or co-factors involved in the 3 major proteolytic systems so far identified in skeletal muscle (*eg*, cathepsin D, a lysosomal protease; m-calpain, a Ca<sup>2+</sup>-dependent protease;



**Fig 1.** Rates of proteins turnover in septic (■) and pair-fed (□) rats. Values are means ± SD.  $p < 0.005$  vs pair-fed (L Voisin *et al*).

ubiquitin and the C8 proteasome subunit, both involved in the ATP-ubiquitin-dependent and proteolytic pathway) were measured in tibialis anterior muscles by using Northern blot procedures, as previously described by Taillandier [Taillandier (1993) Thèse de Doctorat de l'Université Blaise Pascal, Clermont-Ferrand II, France]. These mRNA levels appear strongly correlated with the rate of protein breakdown in many different muscle protein wasting conditions [Attaix *et al* (1994) *Reprod Nutr Dev* 34, 583-597].

The food intake of infected rats was depressed by 84% during the experimental period, and body weight loss was 20% within 2 d of infection. The mass of the tibialis anterior muscles was 19% lower in septic rats than in pair-fed controls. The rate of protein breakdown in the incubated epitrochlearis muscle of infected animals was 49% higher ( $P < 0.05$ ) than in the control group, but protein synthesis was not significantly depressed (fig 1). There was no variation in mRNA level for cathepsin D in the infected group compared to the control. By contrast, mRNA levels for the m-calpain, ubiquitin and the C8 proteasome subunit were systematically higher in septic animals than in pair-fed controls.

These data demonstrate that skeletal muscle protein wasting observed in septic animals mainly results from increased proteolysis in rigorous conditions of pair-feeding. They also suggest that the coordinate activation of both Ca<sup>2+</sup>- and ATP-ubiquitin-dependent proteolytic pathways is mainly responsible for skeletal muscle protein loss. Increased ATP-ubiquitin-dependent proteolysis has been reported in all muscle protein wasting