Nutritional effects on the calpain system in skeletal muscle of sheep

B.C. THOMSON, V.H. ODDY[1] AND R.D. SAINZ[2]

School of Agriculture and Forestry, University of Melbourne, Parkville, Victoria 3052, Australia.

ABSTRACT

The calpain system consists of two enzymes and their endogenous inhibitor, calpastatin, and is involved in the breakdown of myofibrillar protein in skeletal muscle. We have investigated the relationship between the calpain system and protein degradation in sheep under three different feeding regimes. Calpains were separated from fresh muscle using a DEAE-Sephacel ion exchange column. Calpain I and calpastatin activities were higher in the restricted group than in the *ad-libitum* group. Fasting increased calpastatin but had no effect on the calpain I activity. There was a positive relationship between the calpastatin : calpain I ratio and protein degradation and a negative relationship with calpain I. This result is at variance with the idea that calpain I activity limits the rate of protein degradation.

Keywords Calpain, nutrition, protein degradation, calpastatin.

INTRODUCTION

Skeletal muscle growth (lean meat production) reflects the net balance between muscle protein synthesis and degradation. Whilst much is known about the regulation of protein synthesis the factors affecting the regulation of degradation are poorly understood. It is quite clear however that nutrition plays a role in controlling protein turnover (Reeds, 1989). How it does so is open to question. One of the systems thought to be involved in the initiation of myofibrillar protein degradation is the calpain system (calcium dependent protease) (Goll *et al.*, 1989).

The calpain system consists of two enzymes (calpain I and II; cal I, cal II; E.C. 3.4.22.17) and their inhibitor, calpastatin (calp). These enzymes are found in the cytoplasm of a wide variety of cell types (Barrett, 1980) and are active at neutral pHs. *In vitro* they degrade proteins only to large peptides (Goll *et al.*, 1989). The calpains act on troponin and tropomyosin but not actin or myosin. Therefore they have the appropriate properties required for the initiation of protein degradation in skeletal muscle.

Calpain activity has been shown by several workers to be related to nutrition. Total muscle calpain activity increased in fasted rats (Brooks *et al.*, 1985). In milk fed lambs calpain I and calpastatin activities increased and the calpastatin to calpain I ratio declined as feed intake increased (Thomson *et al.*, 1991). Sainz (unpublished) found that calpastatin increased with feed intake and growth rate in pigs. Where there have been studies suggesting that nutrition has little effect on calpains (Higgins *et al.*, 1988), these have used frozen muscle samples. Freezing, however, reduces the recovery of calpastatin (Koohmaraie, 1990) and calpain I (Sainz *et al.*, 1992).

This preliminary study investigated the relationship between the calpain system and protein degradation in lambs on different nutritional regimes.

MATERIALS AND METHODS

Thirteen Hyfer (Merino Dorset cross, Fogarty 1984) wether lambs approximately 5 months old with mean (± sem) body weight 31.5 ± 0.6 kg at the time of experiment, were used for this study. They were weaned at approximately 3 months of age, trucked to the laboratory and fed unrestricted amounts of a pelleted diet, consisting of 70% lucerne hay and 30% triticale grain (900 g dry matter / kg, 140 g crude protein, 10.5 MJ ME / kg dry matter) until 2 weeks prior to experiment.

The lambs were allocated to 3 groups based on amount of feed on offer. One group of 7 lambs were fed 900 g/d (restricted) and the other group of 6 were allowed ad libitum access to feed throughout except for 3 lambs (fasted) from which feed was removed 48 hr before the experiment. The mean (± sem) intake in the *ad libitum* group was 1590 ± 124 g/d. During the measurement period, and for 2 weeks before, food was delivered to the fed lambs by continuous feeders. Water was available at all times.

Kinetics of protein turnover (degradation, gain and synthesis) were calculated from measurement of arteriovenous difference in phenylalanine (Phe) and its specific radioactivity (Barrett *et al.*, 1987, Oddy *et al.*, 1988). The lambs were prepared with indwelling catheters (1.0 x 0.8 mm polyethylene, Dural Plastics, Australia) in their femoral arteries 10 days prior to an experiment (Oddy *et al.*, 1987). Catheters (1.5 x 1.0 mm polyethylene) were inserted into the deep femoral vein of each leg and a jugular vein 2 days prior to an experiment.

Each experiment consisted of continuous infusion of l-[ring 2,6] 3H Phe (Amersham, UK) for 8 hrs at ~ 8.5 kBq/min. Hind limb blood flow was measured in the last hour of infusion. At the end of the blood flow measurement the lambs were killed by overdose of barbiturate and samples of the biceps femoris and vastus lateralis muscles rapidly removed and placed on ice for immediate analysis of calpain activities.

Calpains were analysed by an adaptation of the method of Wheeler and Koohmarie (1991). Muscle was homogenised in Tris buffer (40mM Tris, 10mM EDTA, 0.2% Triton X-100, 10mM ß mercaptoethanol, MCE: pH 7.5), loaded onto a DEAE Sephacel column (1cm by 10cm), washed with buffer A (40mM tris, 0.5mM EDTA, 10 mM MCE; pH 7.5). Calpastatin was eluted with buffer A + 100mM NaCl, calpain I with 200mM and calpain II with 400 mM buffer A. Fractions were assayed using

[1]NSW Agriculture, Elizabeth MacArthur Agricultural Institute, Camden, NSW 2570, Australia.
[2]Present address Department of Animal Science, University of Calfornia, Davis, CA 95616, USA.

casein as the substrate (Hammersten, US Biochemical). One unit of calpain activity was defined as as the amount of enzyme that catalyzed an increase of one absorbance unit at 278nm in 60 minutes at 25°C. Calpastatin was assayed as the inhibition of calpain II proteolytic activity (Wheeler and Koohmaraie, 1991).

RESULTS AND DISCUSSION

Calpastatin and calpain I activities were higher in the animals on 900g/d than those on *ad libitum*. Fasting increased the activity of calpastatin but had no effect on calpain I activities compared to the animals fed *ad libitum* (Table 1).

TABLE 1 Effect of nutrition on calpain I and calpastatin (U/kg protein) activities in Hyfer lambs. Values shown are means with pooled standard deviation (SD).

	ad lib	restricted	fasted	SD
Calpain I	9.7[a]	14.6[b]	9.2[a]	3.82
Calp	29.0[a]	43.2[b]	52.7[c]	4.87
Calp/cal I	2.5[a]	3.0[a]	5.5	1.93
PD*	473[a]	372[a]	650[b]	154

[abc]different superscripts differ (P<0.05)

* PD, muscle protein degradation (nmol Phe/kg/min)

Across treatments, protein degradation was postively related to the calpastatin: calpain I ratio and negatively related to calpain I (E1, E2). The regression between degradation and calpastatin was non significant.

Degradation = 218 + 149 calp:cal I (P<0.001) (E1).

Degradation = 678 - 18.3 calpain I (P<0.05) (E2).

The ratio of calpastatin to calpain I was significantly elevated in fasted animals which also had the highest rate of protein degradation. The ratio provides an indication of how the balance between the enzyme calpain I and its inhibitor calpastatin has altered. Protein degradation is expected to increase when the ratio decreases. This is contrary to these results. If calpain I was involved in the initiation of protein degradation then increased protein degradation rates should have been associated with higher calpain I activities or reduced calpastatin activities ie a lower calpastatin to calpain I ratio.

Our results show that the calpain system is affected by nutrition, but its relationship with protein degradation in muscle is unclear. Protein degradation, as measured in the hind limb preparation used here, is from all sources in the muscle - the major fractions of which are myofibrillar, sacroplasmic and connective tissue. In rats, nutrition differentially affects myofibrillar and sacroplasmic protein degradation (Lowell *et al.*, 1986; Hasselgren *et al*, 1989). We have not yet been able to determine if the differences in protein degradation in response to nutrition in the lamb are due to changes in differential degradation of myofibrillar or non-myofibrillar components of muscle.

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