Myofibrillar Protein Status of the Gastrocnemius in Male Rats: Effect of Mild Undernutrition

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Summary
The aim of this work was the determination of the myofibrillar protein profiles in the fed and the mildly underfed rat. Sixteen male rats were divided into 2 groups: CR (control) fed *ad libitum* and MR (mildly undernourished) fed 75% of energetic maintenance needs. The animals were sacrificed at day 23 and the gastrocnemius muscle was taken for myofibrillar protein characterisation. The myofibrillar protein profiles were found to be very similar in the two groups revealing the lack of preferred catabolism of myofibrillar proteins and consequently that the muscle structure is maintained even in situations of mild undernutrition.

Introduction
Livestock production in tropical areas is seriously affected by undernutrition caused by the season's poor nutritive value of pastures during the dry season. In fact this is frequent, in non-intensive systems due to the lack of supplementation such that a seasonal weight loss of up to 40 % in beef and mutton herds can be expected (*Clariget et al, 1998*). Undernutrition is consequently a serious limitation to animal production in tropical areas significantly reducing productive performances (*Wilson, 1987*).
The above-mentioned weight loss is characterised by the mobilisation of body depots, especially fat and to a lesser extent protein (*Belkhou et al, 1991*). Muscle, and therefore protein, breakdown occurs in order to use the resulting amino-acids as an energy source, which tends to cause a various physiological responses at muscle level (depending on several factors such as the species studied or the severity of food restriction).

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Undernutrition significantly affects several muscle characteristics (*Bedi et al, 1982; Crouse et al, 1984; Mosoni et al, 1999; Ameredes et al, 1999*) mainly as a result of the increase of degradation levels (*Wassner et al, 1977; Ogata et al, 1978*) but also the decrease of protein synthesis (*Fong et al, 1989; Van Eanaeme et al, 1998*). Increasing whole-protein degradation levels are expected to influence not only total myofibrillar protein breakdown but also each one of the myofibrillar proteins (*Fong et al, 1989*). This paper aims to characterise the physiological components of undernutrition, using myofibrillar protein profiles in order to establish, in rats, a model for draught-induced undernutrition periods previously mentioned. Depending on the characteristics of the climate, seasonal undernutrition caused by lower rainfalls can be relatively mild as seen for instance in the Mediterranean climates or in sub-tropical regions. Results obtained with this experiment can hence be extrapolated to species of domestic animals such as sheep or goats that undergo relatively mild undernutrition periods below maintenance levels.

Material and Methods
Sixteen adult male rats (380 g) were obtained from the Wistar Colony maintained at the Gulbenkian Science Institute (Oeiras, Portugal) and divided into

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two weight-matched groups (n = 8): CR (Control) and MR (mildly underfed). Group CR animals were fed *ad libitum*, while MR group animals were restricted to 75 % of the energetic maintenance requirements at the beginning of the experiment (5 g / day). Both groups were fed on milled standard *CRM Interfauna Ibérica* chow (16401 J / g, 17.5 % total protein, 5.3 % ash, 4 % rude fibre and 2.7 % fat).

The animals were housed in a sound-proof room at 24ºC with 8 hours dark per 16 hours of light using individual metabolic cages that allowed separation of faeces and urine (*Cardoso & Stock, 1998*). Nitrogen balances were conducted (*Cardoso & Stock, 1998*) at days 0 and 23 of the experiment in order to characterize the level of undernutrition and hence estimate body depletion conditions. After quantification, faeces were dried to constant weight and the dry matter percentage determined. A sample of the dried faeces was homogenized and used for crude protein and gross energy determinations using standard methodologies (*Cardoso & Stock, 1996*). Briefly, crude protein was determined in a Tecator Digestion System 40 in concentrated sulphuric acid. Distillation was performed in a Tecator Kjeltec System Distilling Unit using 13N Sodium Hydroxide. tritation was conducted using 0.0714N HCl for Nitrogen content determination. Crude protein was determined by multiplying N conten by 6.25. Urine samples were processed using similar methodology. Nitrogen balances were calculated by subtracting total protein of the faeces and urines from protein ingested.

Animals were weighed every two days and feed intake registered daily. After 23 days of experimental period, the animals were anaesthetised (intraperitoneal injection of Sodium Pentothal, 0.5 %) and sacrificed.

The eviscerated carcass was cleaned and “dressed” (decapitation, amputation at the tibio-tarsal, radio-cubit-carpal and sacro-coccigenal joints), and the gastrocnemius muscle collected and kept at -75ºC for further analysis. Gastrocnemius muscle is considered a model to study muscle plasticity and characteristics under stresses (*Alford et al., 1987*). Additionally it is a muscle of easy access, which renders subsequent manipulations easier.

The remaining carcass was frozen, freeze-dried to constant weight and milled and the resulting powder was used for the crude protein content determination as previously described. Total fat was determined by subtracting moisture and crude protein from carcass fresh weight as described (*Carter et al, 1991*). Gross energy was conducted using a Parr 1261 calorimetric bomb (Parr, Moline, Illinois). Total quantities of fat, protein and energy were determined by multiplying the contents by total carcass weight.

Gastrocnemius muscles were prepared using the methods described by Parrish Jr. et al (1973) for the determination of myofibrillar protein extract. Electrophoresis of myofibrillar proteins was done on SDS-PAGE electrophoretic gels at 160 volts. Gels were fixed with methanol and acetic acid for 30 min., washed with distilled water and coloured with 1.0g per litre Coomassie R350. Gels were digitised and analysed for band areas of the following myofibrillar proteins: Myosin Heavy Chains, Protein C, α-Actinin, Tropomyosin + Troponin T, Actin and also the injected pattern Bovine Sera Albumin, in a Kodak Digital Science Gel analyser, according to methods described by (*Claeys et al 1995*).

Results of both groups were compared by ANOVA Single factor. Results were considered significantly different when $p<0.05$. Data are presented as mean values +/- SEM (Standard Error of Mean).

This experiment and laboratory animal handling procedures were approved by the Scientific Council of the Faculty of Veterinary Medicine (Technical University of Lisbon, Portugal).

Results and Discussion

Table 1 refers to the initial and final live weight of rats of both groups, as well as growth and the relative evolution of liveweight and cumulative feed intake. CR animals increased their live weight by 78.80g (a 21 % increase relative to the beginning of

Table 1 - Growth and evolution of rat live weight

	CR	MR
Live weight day 0 (g)	363.19 (4.77)	364.84 (7.98)
Live weight day 23 (g)	441.99^{A} (8.33)	269.84^{B} (8.67)
Growth (g)	78.80^{A} (9.71)	-99.01^{B} (10.25)
Live weight day 23 (as %LW day 0)	121	73
Cumulated Feed Intake (g)	709.18^{A} (20.95)	257.28^{B} (0.48)

^{A,B} Rows with different superscripts indicate statistical significance ($p<0.05$);
Standard Error of Mean values in parenthesis

the experiment), while MR lost 99.01g (a 28 % decrease). Live weight at day 23, growth, as well as live weight evolution registered, were significantly different in both groups.

Nitrogen balances are presented in Table 2. At the beginning of the experiment, balances were positive and similar for both groups. At the end of the experimental period, balances were negative for the MR group as expected and as a consequence of the undernutrition experienced by this group.

An evolution in live weight and negative nitrogen balances, as experienced by the MR group animals, led to subsequent alterations in the carcass composition. As shown in Table 3, all the characteristics studied were significantly higher in the CR animals, with the exception of moisture percentage and crude protein percentage.

Based on previous results, animals in the MR group will be under a situation of undernutrition that has consequences on the depletion of body depots, namely fat. The subsequent metabolic response of this will be depletion of the organism's muscle proteins (*Belkhou et al, 1991*), a situation that the MR animals are clearly experiencing as shown by the decrease in the total quantity of protein.

In such a context, it is interesting to know how muscle protein is affected by catabolism caused by undernutrition. We have conducted such research at the level of the gastrocnemius muscle in the rat by

Table 2 - Nitrogen Balance

	CR	MR
Nitrogen Intake day 0 (g)	0.89 (0.03)	0.83 (0.02)
Nitrogen in the faeces day 0 (g)	0.15 (0.01)	0.17 (0.01)
Nitrogen in the urine day 0 (g)	0.47 (0.02)	0.43 (0.04)
Nitrogen Balance day 0 (g)	0.26 (0.03)	0.24 (0.04)
Nitrogen Intake day 23 (g)	0.76^{A} (0.07)	0.20^{B} (0.01)
Nitrogen in the faeces day 23 (g)	0.13^{A} (0.01)	0.03^{B} (0.01)
Nitrogen in the urine day 23 (g)	0.38^{A} (0.03)	0.27^{B} (0.02)
Nitrogen Balance day 23(g)	0.25^{A} (0.06)	-0.1^{B} (0.03)

^{A,B} Rows with different superscripts indicate statistical significance ($p<0.05$);
Standard Error of Mean values in parenthesis

Table 3 Carcass Composition

Characteristics	CR	MR
Fresh Weight (g)	218.91[A]	145.33[B]
	(4.19)	(3.86)
Dry Matter (%)	30.32[A]	26.05[B]
	(1.09)	(.45)
Humidity (%)	69.68[A]	73.95[B]
	(1.09)	(.45)
Crude Protein	55.67[A]	75.90[B]
(% of dry matter)	(1.89)	(1.22)
Fat Content	44.33[A]	24.10[B]
(% of dry matter)	(1.89)	(1.22)
Gross Energy	25.3[A]	19.4[B]
(kJ / g MS)	(.032)	(.08)
Total Protein (g)	36.76[A]	28.78[B]
	(1.42)	(1.20)
Total Fat (g)	29.86[A]	9.12[B]
	(2.39)	(.50)
Total Energy (kJ)	1686.15[A]	738.05[B]
	(90)	(36)

[A,B] Rows with different superscripts indicate statistical significance ($p<0.05$);
Standard Error of Mean values in parenthesis

electrophoresis. Band areas for the myofibrillar protein Myosin Heavy Chains, Protein C, α - Actinin, Tropomyosin + Troponin T and Actin are depicted in Figure 1. Such data were obtained from gels such as the one depicted in Figure 2. Both groups registered very similar band areas for each of the studied proteins. No significant difference was registered between MR and CR animals. Such results are in accordance with previous results from our experiments (*Almeida et al, 2002*) regarding myofibrillar protein profiles in the rat gastrocnemius muscle under severe undernutrition (34 % weight loss) and contrasts with the results from (*Bates et al 1983*) and (*Clark & Wildenthal 1986*) that point to a decrease in myosin synthesis in situations of more severe undernutrition than that mentioned in this work.

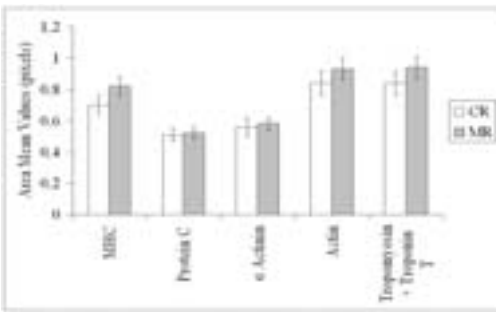

Figure 1 – Myofibrillar Protein Profiles in the gastrocnemius of laboratory rats following mild undernutrition. MHC – Myosin Heavy Chains

The results seem to indicate that in the conditions of this experiment, there is no preferred protein catabolism as a response to undernutrition, thus maintaining myofibrillar protein proportions and also the structure of the gastrocnemius muscle.

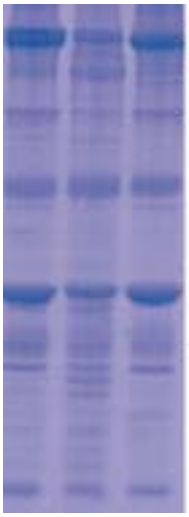

Figure 2 – Example of Myofibrillar protein electrophoresis gel of laboratory rat semimembranous muscle. MHC – Myosin Heavy chains; A – Actin; Pc – Protein C; Aa - a Actinin; TT – Tropomyosin + Troponin T

However, an increase in the level of undernutrition would probably lead to a rupture in muscle structure, with changes in the levels of proteins such as Myosin Heavy Chains, as reported (*Bates et al., 1983; Clark & Wildenthal, 1986*).

Interestingly, alterations in the myofibrillar protein profiles have been reported in the semimembranous muscle of male *Capra hircus* under mild undernutrition of 20 % weight loss (*Almeida et al, 2004*). Such results might indicate that the responses to undernutrition, regarding myofibrillar proteins, vary between species and muscle studied. It seems therefore interesting to extend this research to other muscles in order to have a full picture of the influence of undernutrition on myofibrillar protein profiles.

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