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Muscle Cell Growth in Protein Deficient Rats Following Administration of Sheep Red Blood Cells

E. Muñoz-Martínez*, M.T. Unzaga, A. Agis and M.E. López-Oliva

Sección Departamental de Fisiología Animal,
Facultad de Farmacia. U.C.M.
28040 Madrid (Spain)

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In order to observe the effects of sheep red blood cells (SRBC) administration on the muscle cell growth in malnourished states, adult male Wistar rats (135 ± 10 g 10 animals per group) subjected during 30 days to 1 % and 10 % protein diets, were injected (i.v.) either 15.5 × 10^8 sheep red blood cells or 0.5 ml saline/100 g b.w. after 20 days of experiment. On the 10^{th} day after injection the animals were sacrificed and the gastrocnemius muscle was removed, weighed and homogenized. The supernatant fluids were used to evaluate muscle protein, DNA and RNA rates and acid DNase activity. All parameters were depleted in malnourished rats, indicating a muscle cellular atrophy as well as a decrease in muscle protein synthesis per DNA-unit. Muscle hyperplasia and hypertrophy were found in antigenically stimulated rats fed 10 % protein against non-stimulated control. In contrast, muscle growth in protein-deficient rats SRBC-treated was unmodified when compared to non-stimulated malnourished muscle, although RNA functionality seems to be enhanced (RNA/DNA). These data suggest that a redistribution of essential nutrients occurred for muscle growth adaptation rather than for defensive mechanism.

Key words: Muscle growth, Protein malnutrition, SRBC.

Protein metabolism during infection, sepsis and trauma is characterized by a dominance of catabolic over anabolic processes manifested by absolute losses of nitrogen and wasting of lean body mass (10).

This response appeared to be related to the severity of injury (7), the age of the patient, the pre-injury nutritional status of the individual (3) and nutritional intake following trauma (5).

Muscle protein makes a significant contribution to the whole body protein turnover rates. The catabolism of muscle protein, appears to represent the mobilization of a relatively expendible and labile N store to satisfy requeriments of ami-

* To whom all correspondence should be addressed.

noacids and metabolizable energy (20), but these processes require an adequate supply of dietary protein.

In this sense, it has been observed that protein malnutrition results in increased muscle synthesis capacity in antigenically stimulated rats against control (18). By contrast, muscle of stimulated rats fed with restricted diet (50 % of the control), showed an increased protein catabolism in response to stress (17), indicating a high mobilization of muscle proteins that may compromise survival by depletion of whole-body protein reserves.

These changes in the protein synthesis are indicated by the muscle RNA concentration, and according to WATERLOW *et al.* (25) will depend presumably, among other factors, on the total amount of DNA available for synthesis of RNA.

It follows that alterations in the amount of protein synthesis per DNA-unit can occur if there are changes in the RNA/DNA ratio. It suggests that modifications in muscle number cells and their DNA-unit size can modify tissue protein synthesis.

Both processes, malnutrition (16) and immunization (9), are characterized by a body growth retardation phenomenon, but little information is available, however, about these alterations and their precise mechanism to cellular level.

Consequently, the study of adaptive growth changes in the gastrocnemius muscle during the response to stress after suffering adaptation to protein malnutrition is of great interest.

The present work was designed to determine the changes in skeletal muscle cell growth (DNA metabolism) in protein malnourished rats and their reflection on the response to sheep red blood cells (SRBC).

Materials and Methods

Forty male Wistar rats, 8 weeks old weighing 135 ± 10 g were used and divided into two groups. Each group was fed *ad libitum* for 30 days with a 10 % protein diet (casein + D L, methionine; Control (C) group) or with a 1 % protein diet (protein-deficient (PD) group). Table I shows the diet composition.

Each group was divided into two subgroups after 20 days: control saline-treated (CS) and protein-deficient saline-treated (PDS), injected (i.v.) with 0.5 ml saline/100 g b.wt. and control SRBC-treated (CI) and protein-deficient SRBC-treated (PDI), immunized with 15.5×10^8 SRBC/0.5 ml saline/100 g body weight.

The rats were kept at 23 ± 1 °C artificially illuminated for 12 hour light-dark cycle. Food intake and body weight changes were recorded daily for each rat. At the end of experimental period the rats were decapitated and the gastrocnemius

Table I. *Composition of diet (g/100 g dry matter).*

Ingredients	Control	Protein-deficient
Casein	9.8	0.80
Sucrose	38.02	42.52
D, L-Methionine	0.20	0.20
Starch	38.02	45.52
Cellulose	5.00	5.00
Olive oil	5.00	5.00
Sunflower seed oil	0.50	0.50
Salt mixture[1]	3.34	3.34
Vitamin mixture[2]	0.12	0.12
Protein content (g)	10	1
Gross energy (Kcal/ 100 g)	406	404

1. The mineral mix provides (per 100 g of diet) $CaHPO_4$ 1.3 g; $CaCO_3$ 0.8 g; KCl 0.8 g; Na_2HPO_4 0.75 g; $MgSO_4.H_2O$ 18.0 mg; $C_6H_5O_7Fe.3H_2O$ 17.4 mg; $CuSO_4$ 1.5 mg; $ZnCO_3$ 3.0 mg; KIO_3 0.1 mg.

2. The vitamin mix provides (per 100 g of diet) retinol acetate 1.0 mg; cholecalciferol 0.1 mg; tocopherol acetate 7.5 mg; menadione 0.1 mg; thiamine HCl 1.0 mg; pyridoxine HCl 1.0 mg; riboflavin 1.0 mg; nicotinic acid 6.0 mg; calcium pantothenate 4.0 mg; folic acid 0.5 mg; biotin 0.1 mg; cyanocobalamine 0.005 mg; ascorbic acid 7.5 mg; choline bitartrate 0.18 g.

muscle was carefully removed and weighed.

Aliquots of each sample of muscle (40 aliquots) were homogenized (Ultra-Turrax, Stauphen I Breisan) with a cold-buffer, ph 7.4 (0.15 M NaCl, 0.005 M $NaHCO_3$) to make a 20 % (W/V) homogenate. Solids were removed by centrifugation at 500 × *g* for 10 min and the supernatant fluids were used to evaluate total protein and acid deoxyribonuclease (DNAse) activity by LOWRY *et al.* (11) and MC DONALD (13) methods respectively.

The remaining samples of muscle (40 aliquots), previously extracted by trichloroacetic acid, were used to evaluate total DNA and RNA. Total muscle DNA was measured by the diphenylamine method of BURTON (2); total RNA content was evaluated according to SCHMIDT and THANNHAUSER's method (21) modified by MUNRO and FLECK (15). These determinations allow the calculation of the following parameters: cell size, parameter determined by the ratio protein/DNA expressed in mg of protein/mg of DNA, and number of nuclei (NN) that as their former parameters is relatively used in metabolic studies (7) and is determined by calculating the ratio DNA (mg of tissue) × 10^3/6.2, expressed directly in millions, the number of nuclei of corresponding organ. Since skeletal muscle cells are polynucleated, the parameter number of nuclei must be referred to the quantity of

Tabla II. *Variations in body and muscle growth in rats subjected to protein-malnutrition and/or sheep red blood cells (SRBC).*

Body weight gain, food intake and weight, protein content, DNA and RNA rates, number of nuclei (NN), cell size, acid DNase activity and RNA/DNA ratio of gastrocnemius muscle of rats fed *ad libitum* over a period of 30 days on protein diets containing either 10 % protein (control) or 1 % protein (protein-deficient) subjected to SRBC or saline administration (mean ± SEM, n = 10).

Diet	Control (C)		Protein-deficient (PD)	
	Saline (CS)	SRBC (CI)	Saline (PDS)	SRBC (PDI)
Initial BW (g)	135.06 ± 2.52[a]	134.60 ± 1.87[a]	138.56 ± 2.56[a]	135.21 ± 1.92[a]
Final BW (g)	253.10 ± 4.15[a]	262.04 ± 4.41[b]	99.98 ± 2.95[c]	95.91 ± 1.78[c]
Body weight gain (g)	118.05 ± 3.31[a]	127.44 ± 1.70[b]	−38.58 ± 3.62[c]	−42.10 ± 1.22[c]
Food intake (g/day)	18.43 ± 0.36[a]	19.91 ± 0.31[b]	7.89 ± 0.28[c]	7.22 ± 0.25[c]
Muscle weight (g)	1.90 ± 0.21[a]	1.98 ± 0.22[a]	0.74 ± 0.03[b]	0.70 ± 0.03[b]
Protein (mg/organ)	97.34 ± 1.74[a]	198.41 ± 6.84[b]	30.78 ± 1.61[c]	30.90 ± 1.33[c]
DNA (mg/organ)	6.55 ± 0.25[a]	7.73 ± 0.54[b]	5.00 ± 0.42[c]	4.03 ± 0.27[c]
DNA (mg/g)	3.44 ± 0.26[a]	3.90 ± 0.51[a]	6.75 ± 0.44[b]	5.75 ± 0.78[b]
RNA (mg/organ)	4.95 ± 0.23[a]	5.20 ± 0.23[a]	1.34 ± 0.21[b]	1.67 ± 0.08[c]
RNA (mg/g)	2.55 ± 0.35[a]	2.62 ± 0.37[a]	1.81 ± 0.25[b]	2.38 ± 0.27[c]
NN [DNA (mgx10^3)/6.2]	1056 ± 41[a]	1246 ± 87[b]	806 ± 68[c]	651 ± 43[c]
Cell size [Protein (mg/ organ) / DNA (mg/organ)]	15.07 ± 0.59[a]	26.81 ± 1.75[b]	6.15 ± 0.43[c]	7.66 ± 0.29[c]
Cell size [muscle weight (g)/NN]	1.83 ± 0.07[a]	1.65 ± 0.11[a]	0.96 ± 0.09[b]	1.10 ± 0.05[b]
Acid DNase (U/organ)	51.74 ± 1.82[a]	55.86 ± 4.32[a]	12.24 ± 1.40[b]	9.96 ± 0.78[b]
Acid DNase (U/mg prot.)	0.53 ± 0.02[a]	0.28 ± 0.02[b]	0.40 ± 0.06[c]	0.32 ± 0.04[d]
Acid DNase/DNA	7.90 ± 0.28[a]	7.23 ± 0.56[a]	2.45 ± 0.28[b]	2.47 ± 0.19[b]
RNA/DNA	0.73 ± 0.04[a]	0.67 ± 0.03[a]	0.27 ± 0.02[b]	0.41 ± 0.02[c]

a, b, c, d Means ± SEM in the same row not sharing a common superscript differ at $p > 0.05$, for comparison between saline-treated subgroups, and saline and experimental (SRBC) groups.

sarcoplasma corresponding to a single nucleus; other ratios utilized were cell size, expressed by muscle weight (g)/NN, and RNA/DNA, (mg of tissue).

All results were expressed as means ± SEM. The results were compared using the Student's t-test (23). Differences were accepted as significant when $p \leq 0.05$.

Results

To determine the effect of protein deficiency, the saline-treated subgroups of both diets were compared (table II). Unless an increase in relative DNA rate (mg/g), was found protein malnutrition led to a fall in the remaining parameters evaluated. Thus, decreases occurred in food intake and body weight. The final body weight was 60 % lower than control and decreases to 27 % compared with initial body weight, resulting in negative body weight gain. Marked reductions in the muscle weight (61 %), muscle protein (68 %), total DNA (23 %), number of nuclei (24 %), total (72 %) and relative (29 %) RNA, acid DNase specific activity (24 %) and both, protein/DNA (59 %), and RNA/DNA (63 %) ratios, were observed in PDS rats in comparison to control rats. To determine the effect of SRBC administration, the experimental subgroup was compared with the saline-treated subgroup in each diet. The muscle response to stress caused by antigen administration in the control group led to an increase in food intake (8 %), body weight gain (8 %), muscle protein (104 %), total DNA (18 %), number of nuclei (18 %) and protein/DNA ratio (78 %), as well as a decrease in acid DNase specific activity (47 %) indicating an enhanced body and muscle growth. The remaining parameters tested did not show any significant variation. In contrast to this result no significant differences in protein-deficient SRBC-treated rats in comparison with saline-malnourished rats were found in any of the parameters evaluated except a decrease in acid DNase specific activity (20 %) and an increase in both, total (25 %) and relative RNA (30 %) rates.

Discussion

The malnutrition model used showed that the decrease in the supply of proteins leads to an alteration in the muscle cellular growth that causes changes in the muscle response to stress.

The modifications due to protein deficiency were reflected in a sharp fall in body weight gain, and in a reduction of muscle cell number and size, leading to muscle atrophy. A reduction of rate of production and the total number of DNA-units has been shown in malnutrition (14). Also TRENKLE (24) reported that both, energy restriction and protein deficiency, inhibit DNA synthesis in muscle and that DNA synthesis is more sensitive to these deficiencies than the accumulation of protein.

However, the fact that the actual decrease is higher in protein/DNA ratio than in DNA rate, seems to indicate a fall in the average amount of protein managed by a single nucleus in the experimental conditions. Thus, the deposition of protein was more sensitive to dietary treatment than to the formation of new nuclei and can be related to severity of deficiency. Similar results were found in rats fed with protein-free diet (8).

Furthermore, the decrease in acid DNase specific activity in the same proportion as total DNA, suggests a protector mechanism for muscle DNA rate maintenance during severe protein malnutrition, through a diminished DNA breakdown.

Rates of muscle protein synthesis per DNA-unit were also depressed in malnourished rats against control, according to RNA/DNA ratio. The limitation on protein synthesis, which ultimately must have been responsible for the growth failure can result from either, the insuficient number of

nuclei or lesser activity per nucleus. However, according to WATERLOW *et al.* (25) when growth is suppressed entirely and losses of protein are induced, DNA-unit size falls and existing nuclei are less active for protein synthesis.

The administration of SRBC produced higher food intake and body weight gain in CI rats against CS rats, as happened in well-nourished albino rats infected with *Staphylococcus aureus* (22).

In contrast to muscle atrophy phenomenon associated with febrile infection or trauma (1) and the decrease in protein content and DNA rate found in arthritic gastrocnemius muscle induced by adjuvant (19), the administration of SRBC produced an increase in number and size of muscle cell.

This disparity in the results could be attributed according to CLAGUE (4) to the response to different disease states or to a low degree of trauma. Furthermore, the decrease in acid DNAse specific activity led to a low DNA breakdown, which might be related to DNA rate. Moreover, decreased DNA degradation may reveal a high cell division capacity, linked to the enhanced muscle growth. In this sense high values in acid DNAse activity have been demonstrated to be related to lower rate of cell multiplication (2).

However, the main effect of SRBC administration on muscle growth of control rats, seems to be the increase in cell size, expressed by protein/DNA ratio. This result suggests the existence of a correlation between the muscle hypertrophy post-trauma with a possible decrease of muscle protein breakdown, that determines the muscle protein intracellular accumulation in CI rats against CS rats, since rates of muscle protein synthesis per DNA-unit were not modified (RNA/DNA).

The fact that both, body and muscle cellular growth, remained unmodified in the experimental group submitted to protein malnutrition, can be considered as an index of the impairment of the muscle response to new stress. However, the decrease in acid DNAse specific activity (20 %) suggests a triggering of muscle adaptation, in order to conserve muscle DNA rate to control level (PDS), through a DNA disminished degradation. This response can determine a protector mechanism for muscle growth related to higher cell division capacity (12).

Nevertheless, RNA functionality seems to be enhanced since both, muscle protein synthesis per DNA-unit and muscle protein synthesis capacity, together with an inability to increase muscle protein degradation (18), were increased in PDI rats. Similarly, in malnourished rats following leg fracture a greater *in vitro* activity of muscle ribosomes has been reported (26).

In conclusion, muscle growth of rats fed protein-deficient diets, as compared to well-fed rats has been shown to exhibit an altered adaptation to SRBC administration. A redistribution of essential nutrients seems to occur, contributing to make protein available for muscle growth maintenance at the expense of a precarious defensive mechanism.

Resumen

Se estudia el efecto de la administración antigénica sobre el crecimiento celular del músculo esquelético, en ratas sometidas a malnutrición proteica. Se someten 40 ratas Wistar (135 ± 10 g) a dos niveles proteicos en la dieta, 1 y 10 % de proteína (caseína + D,L-metionina), durante 30 días. Los animales de cada grupo se inyectan (i.v.) con ClNa 9 ‰ (0,5 ml/100 g de peso) sola o con 15,5 × 10^8 eritrocitos de carnero, a los 20 días de experimentación. Tras extraer el músculo gastrocnemio, se determina en homogeneizado la tasa de proteínas, de DNA, de RNA y la actividad del enzima DNAsa ácida. La malnutrición proteica aguda determina la disminución de todos los parámetros evaluados, lo que da lugar a la atrofia del músculo y al decrecimiento en la síntesis proteica muscular por unidad de DNA. La ad-

ministración de eritrocitos de carnero en ratas control (10 % proteína) produce un fenómeno de hiperplasia e hipertrofia muscular comparada con las no estimuladas. Por el contrario, en ratas malnutridas, la estimulación no parece modificar el crecimiento celular deficiente en proteínas, aunque la capacidad de síntesis por unidad de DNA parece incrementarse. De los resultados se deduce que los mecanismos defensivos se subordinan a la función de crecimiento en la respuesta muscular al estrés.

Palabras clave: Crecimiento muscular, Malnutrición proteica, Eritrocitos de carnero.

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