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Muscle and Liver Protein Synthesis and Degradation in Growing Rats Fed a Raw Field Bean (*Vicia faba* L.) Diet

M. Goena, S. Santidrián*, F. Cuevillas and J. Larralde

Departamento de Fisiología Humana
Facultad de Medicina
Universidad de Navarra
31008 Pamplona (Spain)

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Body weight gain, food intake, gastrocnemius muscle and liver weight, protein and RNA content, as well as the fractional rates of muscle and liver protein synthesis (k_s, according to the method of constant infusion of L-[^{14}C]tyrosine), growth (k_g) and degradation (k_d), along with RNA activity (g of protein synthesized per day/g RNA) of both organs, were determined in growing male rats fed *ad libitum* over a period of 10 days on 18.7 % protein diets containing either casein (5 % of methionine added) (control) or the raw legume field bean (*Vicia faba* L.) as the sole sources of protein. It has been found that as compared to control rats, those fed the raw legume diet exhibited a significant reduction in the rate of growth, muscle RNA, k_s, k_g, k_d and RNA activity, and a significant increase in liver k_s, k_d and RNA activity. All differences were statistically significant at least at the 5 % level. The possible nature of these findings is discussed.

Key words: Muscle, Liver, Growth, Legumes, Protein metabolism.

Legumes are widely cultivated in order to obtain a relatively good and inexpensive protein supply (9); however, the utilization of these plants both in human and animal nutrition is limited by the action of antinutritive substances contained in the raw seeds (16, 20), as well as by the low sulphur amino acid content of the legume protein (31). Although considerable attention has been paid to the nutritional effects caused by these substances in farm animals as well as in humans, there is little available information on the effects of legumes on protein metabolism. Previous studies carried out in our laboratory have shown that both rats and chickens fed different raw legume diets exhibited a marked increase in the activity of a number of hepatic amino-acid-degrading enzymes (8), as well as increased muscle and liver proteolytic activity (30). We have also found

* To whom all correspondence should be addressed.

several changes in the composition of the skeletal musculature of growing animals fed raw legume diets; these changes were basically reflected by increases in the non-protein nitrogenous fraction and a lessening in the sarcoplasmic nitrogenous fraction of the skeletal musculature (28, 29).

The objective of this work was to further investigate the effects of feeding growing rats a diet in which the raw legume field bean (*Vicia faba* L.) —extensively cultivated in many Mediterranean areas— was taken as the sole source of protein on the rate of muscle and liver protein turnover. The method of the constant infusion of [^{14}C]tyrosine was applied. This procedure allows for the estimation of the fractional rates of protein synthesis and, subsequently, protein growth and degradation. Besides, as an additional index of protein synthesis, RNA activity was evaluated in muscle and liver of the experimental animals.

Preliminary accounts of some of these studies have been presented previously (13, 14).

It has been found that the growth inhibition displayed by the legume-fed animals was accompanied by an outstanding reduction in the rate of muscle protein synthesis and a slight increase in the rate of liver protein synthesis.

Materials and Methods

Treatment of animals and [^{14}C]tyrosine infusion procedure. — All measurements were made on male Wistar albino rats (80-85 g initial body weight). Animals were caged individually in a room maintained at 21 °C with light from 06:00 to 18:00 and darkness form 18:00 to 06:00 h. Two dietary groups of 10 animals each were made as follows: one was fed a diet in which casein (Merck) was used as the sole source of protein; in order to compensate for the relatively low sulphur amino acid content of this protein (4), methionine (5 % of the total diet) (Sigma) was added; this dietary group was taken as control. The other group was fed a diet in which powdered raw seeds of the legume field bean *(Vicia faba* L.) were used as the sole protein supply. Diets were isocaloric and were prepared according to AOAC (1) recommendations. The total protein content was about 18.6-18.8 % (N × 6.25). Composition of the diet, as well as that of the raw field bean seeds is given in table I. Both food and water were supplied *ad libitum* to the experimental animals over a period of 10 days. Body weight changes and food intake were individually recorded every day for each rat. Rates of protein synthesis were determined by the constant infusion method with [^{14}C]-tyrosine described by GARLICK and MARSHALL (10) and GARLICK *et al.* (11), except that infusions were into a tail vein. At the end of the feeding period, animals were immobilized by wrapping them in a cloth that allowed normal breathing; an insulin needle (0.4 × 0.16 mm) connected to a polyethylene catheter (Silastic Medial-Grade Tubing, 0.30 and 0.64 mm of in-

Table I. *Diet constituents: entries are g %.* Both control and *Vicia faba* diets were provided *ad libitum*. The mineral and vitamin mixtures were prepared after Rogers and Harper (see ref. 21). *Vicia faba* composition (%): protein, 23.5; ether extract, 0.9; ash, 2.8; crude fiber, 6.9; wet, 10.8; total carbohydrate, 44.9.

	Casein	*Vicia faba*
Casein	20.80	—
Methionine	5.00	—
Raw *Vicia faba*	—	76.00
Saccharose	28.75	8.00
Potato starch	28.75	8.00
Olive oil	4.50	4.00
Cellulose	6.00	—
Mineral mixture	4.50	2.50
Vitamin mixture	1.65	1.60
Total protein (N × 6.25)	18.8	18.6

ternal and external diameter respectively) was inserted into a lateral tail vein. L-[U-^{14}C]tyrosine (Amersham International Limited, UK) (12.5 Ci/mol) was dissolved into 0.9 % NaCl at a concentration of 2.5 μCi/ml and infused (with the help of a Harward Syringe Pump, model 2681, Harward Apparatus Co., Natick, MA, USA, that ensured a constant infusion rate ± 1 %) at 1.2 μCi/h for 6 h. All [^{14}C]tyrosine infusions were begun between 08.00 and 09.00 h. At the end of the infusion, animals were rapidly killed by decapitation. Liver and gastrocnemius muscle from rear limbs were immediately excised, weighed and frozen in liquid N_2. The gastrocnemius muscle was chosen since it is reasonably large and easy to dissect quantitatively as well as being reasonably representative of the mixture of fibre types present in rodent muscle (2, 27).

Measurement of the fractional rates of skeletal muscle and liver protein synthesis, growth and degradation by [^{14}C]tyrosine infusion. — Protein and protein-free supernatant fractions were prepared from muscle tissue and liver as previously described (5, 11). The specific radioactivity of L-tyrosine in protein hydrolysates (radioactivity in bound protein S_B), and in the protein-free supernatant (radioactivity in the free intracellular pool, S_i) fractions were measured by the procedure of GARLICK *et al.* (11). Protein content was evaluated by the method of LOWRY *et al.* (17).

To calculate the rate of protein degradation, it is necessary to estimate the rate of protein growth at the time of [^{14}C]tyrosine infusion; an attempt has been made to define as accurately as possible the growth rate of the protein mass by measuring the changes on the three last days and on the subsequent day to that when the perfusion experiments were performed (i.e., measurement of the fractional rate of protein synthesis). To achieve this objective, two other groups of 12 rats each, analogous to those mentioned above, were used to provide information about the changes in muscle and liver protein mass. Caging, dietary treatment and other experimental details were as already explained. Since the ratio of protein content of the gastrocnemius muscle and liver to body weight (expressed as a percentage) did not significantly change during those days, the daily percentage change in body weight during this interval was used to estimate the fractional rate of protein growth at the time of death.

The fractional rate of muscle protein degradation was calculated by subtracting the fractional growth rate from the fractional synthesis rate (6). Although the precision of these estimates is not as great as the precision of the measurement of protein synthesis, they do allow for the estimation of possible changes in the rate of protein degradation (26).

Muscle and liver RNA content was determined by the orcinol reaction according to the method described by MILLWARD *et al.* (22). Values of RNA activity (the rate of protein synthesis per unit of RNA) were calculated from the RNA/protein ratios and fractional synthesis rate, their units being, therefore, g of protein synthesis/day per g of RNA (27).

Statistical treatment of the data. — Statistical evaluations were carried out by conventional Student's *t* test. Comparisons with the animals fed the casein diet were made. Statistically significant differences were calculated both for $p < 0.01$ and $p < 0.05$.

Results and Discussion

Table II shows that, as previously published (19), feeding growing rats a diet in which the raw legume field bean is the sole source of protein brings about a significant ($p < 0.01$) reduction in the rate of

Table II. *Body weight gain, food intake, gastrocnemius single muscle and liver weight, protein content and total RNA of growing male rats fed* ad libitum *over a period of 10 days on 18.7 % protein diets containing either casein or the raw legume field bean* (Vicia faba *L.*) *as the sole source of protein.*
Entries are mean values (± SEM) from 10 rats in each group.

	Casein	*Vicia faba*
Body weight gain (g/day)	7.6 ± 0.6	4.9 ± 0.7[b]
Food intake (g/100 g body weight)	12.0 ± 0.5	12.2 ± 0.1
Gastrocnemius weight (g/100 g body weight)	0.54 ± 0.05	0.53 ± 0.05
Liver weight (g/100 g body weight)	4.21 ± 0.16	4.59 ± 0.14
Gastrocnemius protein (mg/g of tissue)	184.4 ± 8.2	166.8 ± 12.0
Liver protein (mg/g of tissue)	160.2 ± 4.1	163.8 ± 5.6
Gastrocnemius RNA (mg/g of tissue)	2.70 ± 0.16	1.85 ± 0.17[b]
Liver RNA (mg/g of tissue)	6.71 ± 0.28	6.51 ± 0.16

[b] $p < 0.01$ (Student's *t* test), as compared to control casein-fed rats.

growth in comparison to control casein-fed animals. However, no significant differences were observed in the amount of food intake, when expressed per unit of body mass, between the two experimental groups. This agrees with similar results found in legume-fed birds (30) and shows that the growth inhibitory effect, as well as the metabolic effects discussed later, cannot be ascribed to differences in the amount of energy or protein ingested by the experimental animals; it seems, therefore, that the organoleptic properties of the legume diet did not influence the nutritional and metabolic effects reported in this study. Expressed per unit of body weight, no significant differences were found in the weight or protein content of either gastrocnemius muscle or liver; this fact partly agrees with the results of MARTÍNEZ and LARRALDE (21) who found a slight reduction of gastrocnemius muscle weight in rats fed a diet similar to the one used in this experiment; however, they fed their rats over 45 days, a feeding period much longer than that used in this study. In any case, our results indicate that muscle protein content tended to be smaller than that of casein-fed animals. It is not completely understood why feeding growing rats a raw field bean diet brings about such a marked growth reduction; nevertheless, as will be indicated later, the reduced muscle protein synthesis (probably due to the deficiency of essential amino acid of the legume protein and to the action of the antinutritive factors contained in the raw legume seeds) undergone by *V. faba*-fed rats might be a contributing factor in order to explain such an effect.

Table III shows that in both gastrocnemius muscle and liver, the ratio protein content to body weight, expressed per 100

Table III. *Gastrocnemius muscle and liver protein/body weight ratio (mg/100 g body weight) of growing male rats fed* ad libitum *over periods of 8, 9, 10 and 11 days on 18.7 % protein diets containing either casein or the raw legume field bean* (Vicia faba *L.*) *as the sole source of protein.*
Entries are mean values (± SEM) of three rats in each group.

	Days of feeding	Casein	*Vicia faba*
Gastrocnemius	8	92.2 ± 2.6	96.5 ± 2.4
	9	90.5 ± 1.9	97.5 ± 2.0
	10	91.7 ± 3.5	96.2 ± 2.6
	11	92.8 ± 2.3	97.4 ± 1.8
Liver	8	638 ± 21	709 ± 17
	9	636 ± 20	713 ± 18
	10	637 ± 18	714 ± 18
	11	638 ± 16	715 ± 16

Table IV. *Gastrocnemius muscle and liver S_B/S_I ratio (specific radioactivity of tyrosine bound to protein and in the free intracellular pool, in dpm/μmol), k_s, k_g, k_d (fractions of protein mass synthesized, grown and degraded each day, in % per day) and RNA activity (g of protein synthesized each day per g of RNA) of growing male rats fed* ad libitum *over a period of 10 days on 18.7 % protein diets containing either casein or the raw legume field bean* (Vicia faba *L.) as the sole source of protein.*
Entries are mean values (± SEM) from 10 rats in each group.

		Casein	*Vicia faba*
S_B/S_I ($\times 10^3$)[1]	Gastrocnemius	24.7 ± 2.4	15.6 ± 1.5[b]
	Liver	122 ± 11	145 ± 10[a]
k_s	Gastrocnemius	14.22 ± 0.75	9.94 ± 0.51[b]
	Liver	53.46 ± 4.59	64.20 ± 4.56[a]
k_g	Gastrocnemius	1.24 ± 0.32	0.25 ± 0.06[b]
	Liver	2.56 ± 0.37	2.04 ± 0.29
k_d	Gastrocnemius	12.98 ± 0.74	9.69 ± 0.51[b]
	Liver	50.90 ± 4.87	64.47 ± 5.93
RNA activity	Gastrocnemius	9.99 ± 1.20	8.80 ± 0.68[a]
	Liver	12.30 ± 1.05	16.93 ± 1.19[b]

[a] $p < 0.05$; [b] $p < 0.01$ (Student's *t* test), as compared to control casein-fed rats.
[1] According to GARLICK and MARSHALL (10) and GARLICK *et al.* (11).

g of weight, was maintained relatively constant during the two days prior to the infusion day, the day of infusion and the one after. Since the ratio did not significantly change during these days, the daily percentage change in body weight during this interval was used to estimate the fractional rate of muscle and liver protein growth (6, 27).

As judged by the results shown in table IV, rats fed the raw legume diet, as compared to control casein-fed animals, exhibited a significant reduction ($p < 0.01$) in the fractional rate of muscle protein synthesis (k_s), growth (k_g) and degradation (k_d) (i.e., percentage of muscle protein synthesized, grown or degraded per day). In addition, RNA activity (protein synthesis referred to RNA mass) was found to be significantly reduced ($p < 0.05$) in the legume-fed rats as compared to control animals. Taking into account that skeletal muscle represents a sizeable part of whole body mass, this outstanding reduction in the rates of k_s, $k_g$and k_d may well be an important factor to explain the growth inhibitory effect caused by this legume in the growing rat. It is interesting to note that the rate of synthesis of muscle protein almost equalled the rate of degradation; therefore, the rate of muscle protein growth resulted very small, although it did not reach negative values, probably due to the shorter term feeding period of our experimental design. These findings are quite similar to those obtained by other investigators when studying the effect of protein malnutrition on muscle protein turnover (15, 32). On the other hand, if it is considered that muscle protein mass is regulated primarily through alterations in protein synthesis (23), it could be assumed that either the low sulphur amino acid content of the legume protein (31) or the action of the antinutritive factors contained in the raw seeds (16) negatively interacts with the anabolic process of skeletal muscle protein synthesis. No attempt has been made in order to differentiate the effect of the raw legume on the fractional rates of protein synthesis, growth or degradation of the sarcoplasmic and myofibrillar fractions, as has been done by other investigators in ex-

periments designed to elucidate the effect of glucocorticoids on muscle protein metabolism (27), but these results might partially explain, the reduced sarcoplasmic nitrogenous fraction observed in legume-fed rats and birds (18, 29); this may be so, specially taking into account that in rat skeletal muscle the overall rate of synthesis of sarcoplasmic proteins is higher than that of myofibrillar protein (3), and therefore, it could be altered to a greater extent than that of contractile proteins.

Finally, the same table IV shows that in comparison to the control animals, a significant increase ($p < 0.05$) in the fractional rate of liver protein synthesis (k_s) —as well as in RNA activity ($p < 0.01$)— has been found in the legume-fed rats; however, in these animals liver fractional protein growth (k_g), although not statistically significant, was found to be smaller than that of control rats; consequently, liver protein degradation rate (k_d) tended to be increased in the legume-fed animals; these data may explain the relative liver enlargement observed in field bean-fed rats. It should be noted that the present measurement of liver protein synthesis includes both fixed liver proteins and those exported, and therefore, it is not possible to differentiate in this study whether there is a quantitative specific effect on the rate of protein synthesis in both types of hepatic proteins. On the other hand, it is well known that changes in protein synthesis or breakdown in liver are originated by increased hepatic uptake or output of amino acids coming from non-hepatic tissues (especially, skeletal musculature) (32). As in other catabolic conditions (e.g., glucocorticoid treatment) (27), it may occur that the reduced muscle protein synthesis and degradation observed in legume-fed rats could account for the protein turnover changes observed in the liver of the experimental animals.

The intrinsic mechanism by which all these metabolic effects take place in raw field bean-fed rats remains to be completely understood. As mentioned above, the insufficient amount of some essential amino acids in the legume protein and, specially, the catabolic effects that may be caused by the toxic substances contained in these plants possibly account for these effects. In this sense, it has been reported that phytohemagglutinins, one of the most important antinutritive factors (along with tannins) contained in raw legume seeds, are able to reduce the rate of protein synthesis in lymphocytes (7,12); on the other hand, it is well known that tannins (24), hemagglutinis (18) and lectins (25) do interact with the brush border intestinal epithelium cells, partly blocking the absorption of amino acids and, therefore, reducing the body availability of these and other nutrients. This could force skeletal muscle to movilize its own proteins in order to supply the organism with the essential amino acids that are either lacking in the diet or cannot be absorbed through the intestinal tract.

In conclusion, the results of this study showed that in comparison to control casein-fed rats, those fed a raw field bean diet exhibited along with a growth reduction, a marked reduction in the rate of muscle protein synthesis accompanied by an increase in the rate of liver protein synthesis.

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Resumen

En ratas machos en crecimiento, alimentadas *ad libitum* durante 10 días con dietas que contenían como únicas fuentes proteicas (18,7 %) caseína o harina cruda de la leguminosa *Vicia faba* L., se determinan las variaciones ponderales, el consumo

de alimento, peso, contenido proteico y de RNA del músculo gastrocnemio y del hígado, así como la velocidad de síntesis (k_s, según el método de infusión constante de L-[^{14}C]tirosina), de crecimiento (k_g) y de degradación (k_d) de las proteínas. También se determina en ambos tejidos la actividad RNA (g de proteína sintetizada por día/g de RNA). Los resultados muestran que las ratas alimentadas con la leguminosa cruda presentan una reducción significativa en la velocidad de crecimiento así como en los parámetros musculares RNA, k_s, k_g, k_d y actividad RNA. Sin embargo, en hígado se encuentran incrementos significativos de los valores de k_s, k_d y actividad RNA. Todas las modificaciones indicadas son estadísticamente significativas ($p < 0,05$). Se comenta la posible naturaleza de estos resultados.

Palabras clave: Músculo, Hígado, Crecimiento, Leguminosas, Metabolismo proteico.

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