

# Protein Synthesis in Growing and Adult Rats on Casein and Gluten Nutrition

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## Summary

Male weaning rats were put on a diet with a physiological nutrient combination adjusted for age, milk casein (E/N = 0.79) and wheat gluten (E/N = 0.30) being the sources of protein. The net protein ratio (NPR) was evaluated weekly until 140 days of age. On days 70 and 140, L-(U-<sup>14</sup>C)-tyrosine was administered intraperitoneally and 12 h later specific tyrosine activity was determined in the protein fraction of liver and muscle by measuring the incorporation of the labeled amino acid in order to assess protein synthesis over the corresponding time period. Regression lines describing the relationship between animals' weight, age and protein source suggested that the daily weight increase was 6.99 g between days 30–77, 2.97 g between days 77–105 and 0.64 g between days 105–140. Muscle tyrosine levels in rapidly growing animals aged 70 days were 91.0  $\mu\text{g/g/12 h}$  for casein and 65.6  $\mu\text{g/g/12 h}$  for gluten. Liver tyrosine levels were 336.4 and 189.6  $\mu\text{g/g/12 h}$ , respectively. The differences observed at this age were highly significant. In adult animals (140 days old) there were non-significant differences between tyrosine levels in the casein- and gluten-fed groups. The isotope study clearly showed that protein synthesis was reduced in growing and developing animals on vegetable nutrition, which is deficient in essential amino acids, (especially the limiting amino acid lysine, crucial for the utilization of all other amino acids in peptide chain synthesis). The low rate of amino acid utilization found in animals younger than 105 days is consistent with the findings obtained by the isotope method.

## Key words

Vegetable nutrition – Protein quality – Protein synthesis – Growing and adult rats

## Introduction

To ensure the physiological performance of the body's metabolic processes, food should contain all nutrients in ratios appropriate for different periods of life (FAO/WHO/UNU 1985, NRC 1978). Special consideration should be given during body growth and development to proteins as building blocks of the living matter with a particular emphasis on their quality and adequate intake necessary for protein synthesis. The ratio between protein synthesis *de novo*, on the one hand, and for tissue replacement and body maintenance, on the other hand, is known to change as a function of age (Bressani *et al.* 1973, Krajčovičová-Kudláčková and Dibák 1986, 1989a).

The present paper describes protein synthesis in laboratory rats of two age groups – the animals during a period of rapid growth (70 days of age) and the adult rats (140 days of age). After weaning, the

experimental animals were placed on a long-term dietary regimen with an optimal combination of nutrients, milk casein and wheat gluten being the sources of protein. An isotope procedure was used whereby a labelled amino acid was administered to animals. The specific amount of the amino acid utilized for protein synthesis over a given period of time was evaluated by measuring its incorporation into liver and muscle protein fraction.

## Material and Methods

Experiments were carried out on male Wistar rats (Velaz, Prague) which were weighing  $68 \pm 5$  g. After 5 days' adjustment to our laboratory conditions these weanling animals were placed on a diet with an optimal nutrient composition adjusted for age as

reported previously (Krajčovičová-Kudláčková 1990). The dietary regimen lasted up to the age of 140 days. Protein was derived from either milk casein (Laktos, Prague) with  $78 \pm 2$  % proteins,  $1.3 \pm 0.1$  % fats and  $2.0 \pm 0.1$  % saccharides or wheat gluten (Slovenské škrobárne, Trnava) containing  $70 \pm 2$  % proteins,  $6.0 \pm 0.4$  % fats and  $12.0 \pm 1.0$  % saccharides. The lipid and saccharide content in prescribed protein doses was subtracted from the margarine (ratio of saturated to unsaturated fatty acids 1 : 4) and from wheat starch amounts used to prepare experimental diets. Saccharides comprised sucrose and wheat starch in a physiological ratio of 1 : 6.2 (Krajčovičová-Kudláčková and Dibák 1989b). The protein content of gluten was calculated by multiplying the casein value by a factor of 1.45 (Krajčovičová-Kudláčková 1992) as suggested by the mean ratio of casein and gluten amino acids verified in biological experiments (Table 1). A vitamin mixture was added to food every day before feeding (Henry 1965).

**Table 1**  
The optimal nutrient formula diet

Age (days)	Nutrients (g/100 g food)					
	Proteins		Lipids	Saccha- rides	Salt mixture	Agar
	Casein	Gluten				
30-45	15	22	30	36	4	15-8
46-75	15	22	20	41	4	20-13
76-90	12.5	18	15	46	4	22-17
91-120	11.5	17	11	51	4	22-17
121-140	10	15	10	51	4	25-20

The nitrogen and/or protein (Nx6.25) content of casein, gluten and food was evaluated by the Dumas method (Vondenhof and Schulte 1979). Lipid content of casein, gluten and margarine as well as lipids in the diets were determined by the Soxhlet extraction method whereas the content of saccharides in casein, gluten, sucrose and wheat starch as well as in the diets were evaluated according to Schoorl (Pribela 1978). The nutrient content in the food was found to be within an acceptable error range of 0.3–0.6 %.

Sixty-four rats fed casein and 16 rats fed gluten diets were individually housed in a cage with a grating floor. Food and water were provided *ad libitum*. Food consumption was evaluated daily and animals were weighed once a week. At different periods of experiment, six groups (comprising of 8 animals each) fed the casein diet were put for 7 days on a protein-free diet in order to determine the mean weights loss and to calculate the net protein ratio (NPR) values (Bender and Doell 1957). The respective periods were 35–42 days, 50–56 days, 64–70 days, 78–84 days, 113–119 days and 133–140 days, i.e. at the start of the

experiment, at the time of the first and second isotope probe and once during rapid growth, adolescence and in the adult age. NPR was calculated as  $(A+B)/C$  where A is the weight increase on tested protein (g), B is the weight decrease on protein-free food (g) and C is the protein intake (g).

On days 70 and 140, 8 rats from each dietary group were subjected to the isotope study (Tanaka *et al.* 1987). The animals fasted from 0730 h to 1630 h and were allowed a free access to food between 1630 h and 1930 h. At 1930 h the animals were given an intraperitoneal injection of L-(U- $^{14}$ C)-tyrosine (sterile 2 % ethanol solution with a specific activity of 13.32 GBq/mmol diluted to the applied dosage with thermostated physiological saline) in a dose of 2.9 mg/g body weight in young animals and 2 mg/g body weight in adult animals (Tanaka *et al.* 1987). Twelve hours after isotope administration the rats were sacrificed under ether anaesthesia, their livers were excised and a muscle sample was taken from the hind thigh. About 250 mg liver and muscle tissues were added to 20 volumes of a chloroform-methanol mixture (2:1) as described by Folch *et al.* (1957). Following 24 h chloroform-methanol extraction and subsequent centrifugation (5000 rpm for 10 min), the sediment was treated with 20 volumes of 10 % trichloroacetic acid to prepare a pure protein fraction of the tissue. After centrifugation, the sediment resulting from protein precipitation with trichloroacetic acid was dissolved at 50 °C in Nuclear Chicago Solubilizer (US Patent 3, 506 828). The dissolved protein fraction (1 ml) was transferred to a scintillation flask into which 50–100  $\mu$ l glacial acetic acid (to eliminate chemiluminescence) as well as 10 ml PCS (Phase Combining System) were added. After a complete elimination of solution fluorescence, radioactive carbon was measured by means of the liquid scintillation method using a 1214 Rack Beta Excel counter (LKB, Sweden) with an external standard.

To determine the tyrosine tissue content after decapitation, liver and muscle were hydrolysed for 72 h in approximately 40 volumes of 6 mol/l hydrochloric acid. The tyrosine content was evaluated in tissue hydrolysates using an automated chromatographic procedure (Spackman *et al.* 1958).

## Results

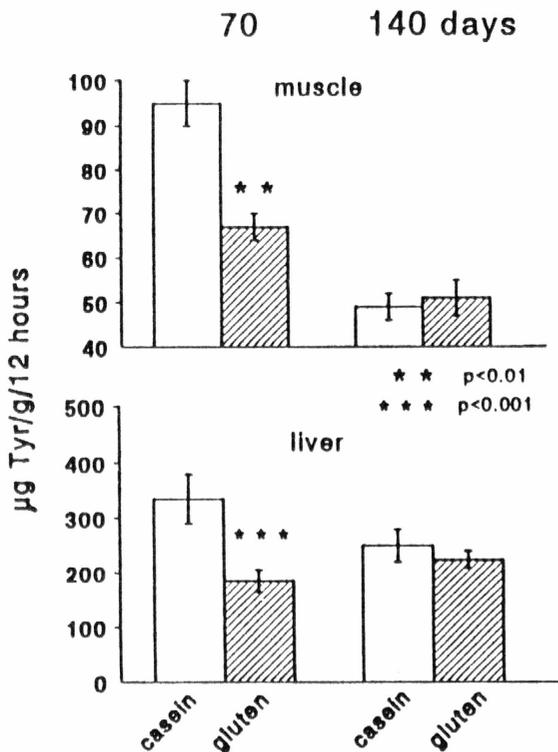
Fig. 1 shows changes in the body weight of rats aged 30 to 140 days receiving diets with an optimal nutrient formula, casein and gluten being the sources of protein. Animals on gluten diet weighed significantly less than those on casein diet during the entire experimental period, with the exception of the two last measurements on days 133 and 140 when the differences were not significant. The growth curve of rats fed casein featured three linear stages: a) days

30-77, b) days 77-105 and c) days 105-140 with respective regression equations:

- a)  $y = 7.00x - 133.7$ ;  $n = 110$ ,  $r = 0.998$ ;  $P < 0.001$
- b)  $y = 2.97x + 172.6$ ;  $n = 40$ ;  $r = 0.985$ ;  $P < 0.001$
- c)  $y = 0.64x + 412.0$ ;  $n = 48$ ;  $r = 0.946$ ;  $P < 0.001$ .

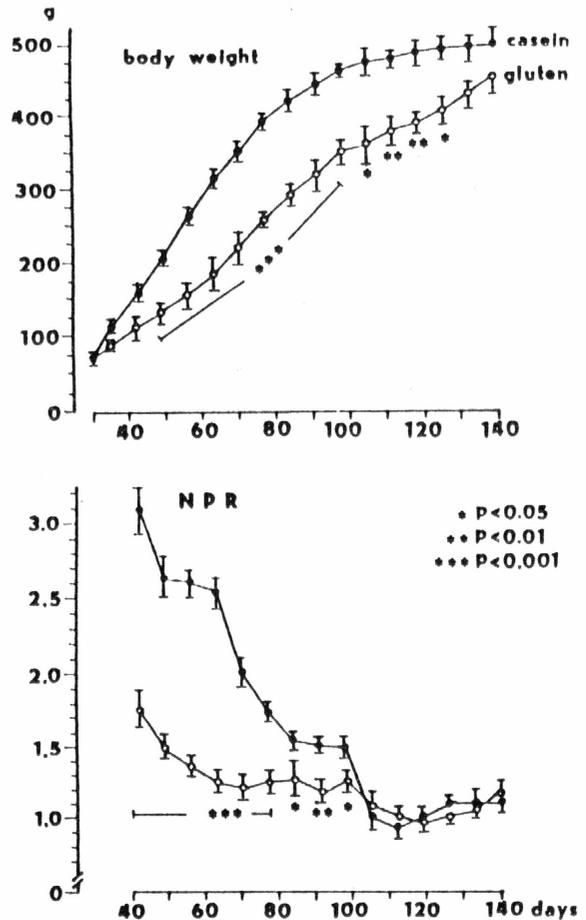
The growth curve of rats fed gluten diet did not display these characteristic segments and the growth observed after 105 days of age was slower. The regression equation corresponding to the growth-age relationship between days 30-105 was  $y = 4.16x - 64.7$ ;  $n = 142$ ;  $r = 0.997$ ;  $P < 0.001$ . The regression equation for the period of growth between days 105-140 was  $y = 2.74x + 73.0$ ;  $n = 48$ ;  $r = 0.942$ ;  $P < 0.001$ . As suggested by regression slopes, rats fed casein increased their weight by 6.99 g a day between 30 and 77 days of age, by 2.97 g a day between days 77 and 105, whereas from the age of 105 days there was a steady growth of only 640 mg a day. Animals on the vegetable diet increased their weight daily by 4.15 g before the age of 105 days and by 2.74 g/day later on.

similar for both protein sources. The NPR decline in gluten-fed animals between days 42-77 was highly significant (28-51 %), the decline between 77 and 105 days of age was 16-23 %.



**Fig. 1** Body weight and the net protein ratio (NPR) in animals aged 30-140 days which were fed a diet with an optimal nutrient formula containing milk casein or wheat gluten as protein sources.

Protein utilization as expressed by the net protein ratio (NPR) (Fig. 1, bottom) was significantly higher in the casein group at the age of 42-105 days, while after 105 days of age NPR values appeared to be



**Fig. 2** Tyrosine (µg Tyr/g/12 h) utilized for protein synthesis in the liver and muscle of rats aged 70 and 140 days that were fed since weaning a diet with an optimal nutrient formula containing milk casein and wheat gluten as protein sources.

Table 2 shows the findings obtained after L-(U-<sup>14</sup>C)-tyrosine administration. The amount of tyrosine utilized for protein synthesis (Table 2, Fig. 2) was calculated from the total amount of the amino acid determined in the liver and muscle after acid protein hydrolysis and from the radioactivity found after 12 h in the liver and muscle protein fraction as a percentage of the total administered isotope dose. Young rats fed casein diet utilized significantly more tyrosine both in the liver and muscle. The increase in the muscle was enhanced by a factor of 1.4 whereas in the liver the factor was 1.7. The tyrosine values found in the muscle of casein and gluten groups at the age of 140 days were similar; the decline observed in the liver of gluten-fed animals was not significant.

**Table 2**

Amount of tyrosine utilized within 12 h for protein synthesis in the liver and the muscle of rats aged 70 and 140 days that were fed since weaning a diet with an optimal nutrient formula containing milk casein and wheat gluten as protein sources

Age days	Tissue	Protein source	Tyr dose applied		Radioactivity		% Tyr dose administered	Tyr levels at decapitation	Tyr utilization for protein synthesis
			mg	dpm x 10 <sup>6</sup>	dpm x 10 <sup>3</sup> /g	% dose applied dpm/g			
70	Muscle	Casein	1021.3 ± 32.1	6.49 ± 0.20	9.58 ± 0.82	0.148 ± 0.012	1.49 ± 0.13	6.11 ± 0.25	91.0 ± 6.2
		Gluten	642.1 ± 50.2	4.08 ± 0.32	9.75 ± 0.62	0.243 ± 0.011	1.51 ± 0.09	4.32 ± 0.13	65.6 ± 3.1*
70	Liver	Casein	1021.3 ± 32.1	6.49 ± 0.20	45.78 ± 2.39	0.707 ± 0.031	7.25 ± 0.37	4.64 ± 0.14	336.4 ± 20.1
		Gluten	642.1 ± 50.2	4.08 ± 0.32	37.28 ± 2.29	0.952 ± 0.090	5.86 ± 0.36	3.24 ± 0.14	189.6 ± 10.4*
140	Muscle	Casein	947.7 ± 25.2	5.73 ± 0.39	6.90 ± 0.54	0.120 ± 0.012	1.13 ± 0.11	4.26 ± 0.16	48.1 ± 3.4
		Gluten	973.8 ± 17.1	5.89 ± 0.21	8.09 ± 0.43	0.137 ± 0.008	1.33 ± 0.08	3.82 ± 0.18	50.8 ± 4.0
140	Liver	Casein	947.7 ± 25.2	5.73 ± 0.39	49.04 ± 2.00	0.855 ± 0.051	8.10 ± 0.48	3.12 ± 0.09	252.7 ± 14.6
		Gluten	973.8 ± 17.1	5.89 ± 0.21	44.72 ± 2.31	0.759 ± 0.046	7.39 ± 0.44	3.01 ± 0.05	222.4 ± 7.2

Data are means ± S.E.M., n = 8; \* significantly different (p < 0.01) from casein group.

## Discussion

The clarification of the role played by a limiting amino acid in protein synthesis (Mitchell 1964) was a turning point in the evaluation of protein quality and its impact on the rate of protein synthesis. The body should receive all essential amino acids, in appropriate amounts and ratios *vis-a-vis* non-essential amino acids, as reported for the reference protein of hen egg or cow milk (FAO/WHO 1973, FAO/WHO/UNU 1985). Only thus can dietary amino acids be adequately utilized for protein synthesis. The ratio of essential to non-essential amino acids (E/N) in a protein or protein mixture should be close to one so that case the aminogram of the consumed amino acid mixture is balanced and fully utilizable for protein synthesis. The E/N value was found to be 0.79 for casein and 0.30 for gluten (Krajčovičová-Kudláčková 1992). The first deficient (limiting) amino acid determines the degree of productive use of all other essential amino acids for anabolic processes as it initiates the synthesis of a peptide chain. The amino acid sequence of the primary structure of synthesized protein is known to be dictated by nucleotide triplets of the genetic code. Other amino acids are incorporated into the peptide chain depending on the available amount of the limiting amino acid.

Methionine or sulphur-containing amino acids are the limiting components of casein. Their content is only negligibly diminished as compared to the egg and the total amino acid content of casein is 94 % of that of the egg (Krajčovičová-Kudláčková and Fecenková 1991). Vegetable proteins have significantly diminished levels of one of the essential amino acids (e.g. grains have less lysine and legumes have less sulphur-containing amino acids), resulting in an unbalanced amino acid composition and a decreased E/N ratio. Gluten contains only 18 % of the reference egg protein lysine, 15 % of casein lysine. Gluten has only 52 % of the total essential amino acid content of the egg and 56 % of that of casein (Krajčovičová-Kudláčková and Fecenková 1991). To adjust the gluten dose in the diet to physiological values for animals of different ages (using a factor of 1.45 as the mean casein/gluten amino

acid ratio), the lysine content increased to 21 %. The gluten aminogram became more balanced *vis-a-vis* casein because essential amino acids increased by 106 mg per gram of proteins (Krajčovičová-Kudláčková 1992).

The amino acid relationship described above were reflected in the isotope study in which the rate of protein synthesis was determined by evaluating the percentage of a radioactive amino acid in the liver and muscle protein fraction of animals on long-term vegetable nutrition as compared with a reference animal protein diet. L-(U-<sup>14</sup>C)-tyrosine was used as a label. According to a recent protein classification system (Chernikov 1986), tyrosine is listed together with phenylalanine and ranked with 7 other amino acids (plus Cys/2) as essential for protein synthesis. As already mentioned, however, a limiting amino acid plays a crucial role so that any amino acid may be randomly selected as such. Rats aged 70 days grow rapidly (6.99 g/day) when fed milk protein as a reference diet. The period between 77–105 days corresponds to adolescence (2.97 g/day) whereas adult animals aged 140 days exhibit a steady growth of only 640 mg/day from 105 days onwards.

Tyrosine levels utilized for protein synthesis and detected in the liver and muscle protein fraction 12 h after isotope administration suggest that young rats (aged 70 days) fed a vegetable (gluten) diet have a significantly lower rate of protein synthesis as compared to control animals on casein diet. Such differences are not detectable in adulthood. These findings unequivocally point out that a long-term exclusively vegetable diet is inadequate during the period of growth and development since it fails to meet nutritional requirements adequately in respect to essential amino acids (namely limiting ones). A slower growth of rats fed gluten diet and diminished amino acid utilization from the vegetable nutrition source which were observed between weaning and 105 days of age when, as suggested by the weight and age relationship in animals on the reference animal protein diet, the adolescence period comes to the end, are consistent with the findings of the isotope study.

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### Reprint Requests

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