

Relative contribution of cysteine and methionine to glutathione content and thyroid hormone levels in the rat

By C. SUBERVILLE, P. HIGUERET, D. TARUOURA AND H. GARCIN

*Laboratoire Physiologie de la Nutrition, Département Alimentation et Nutrition,
Université Bordeaux I, avenue des Facultés, 33405 Talence-Cédex, France*

AND D. HIGUERET

*Laboratoire Biochimie Médicale, Hôpital Pellegrin, Place Raba Léon, 33000 Bordeaux,
France*

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1. For a period of 24 d rats were given diets containing either casein or pea (*Pisum sativum*) protein at two different concentrations (180 and 120 g/kg) without or with cysteine or cysteine + methionine supplementation.
2. The effects of these diets on levels of blood and liver reduced glutathione (GSH) and serum thyroid hormones were studied.
3. When compared with the 180 g casein/kg diet, the 120 g casein/kg diet decreased liver GSH and serum thyroid hormone concentrations. These changes were related to dietary cysteine supply since supplementation induced an increase in these variables.
4. When compared with 180 g pea protein/kg diet, the 120 g pea protein/kg diet decreased liver GSH and serum thyroid hormone concentrations. These changes could not be corrected by cysteine or cysteine + methionine supplementation.

Inadequate food intake produces various changes in hormonal status. For example it is known that vitamin A deficiency (Garcin & Higuieret, 1977; Morley *et al.* 1978; Higuieret & Garcin, 1984*a*) or protein deficiency (see Brasel, 1980) induces changes in thyroid status. It is known also that reduced glutathione (GSH) plays several roles in thyroid hormone physiology, particularly in peripheral metabolism of thyroxine (T_4) (Balsam & Ingbar, 1978; Chopra, 1978; Imai *et al.* 1980; Higuieret & Garcin, 1982) and in 3,5,3'-triiodo-thyronine (T_3) cellular uptake (Higuieret & Garcin, 1984*b*). It is thus of interest to study the effects of sulphur-amino acid-deficient diets on tissue GSH contents and, also, on thyroid hormone changes.

According to Sowers *et al.* (1972) and Stockland *et al.* (1973), cysteine (Cys) and methionine (Met) deficiencies appear in the rat when the dietary supply of Cys and Met are lower than 2.8 and 1.7 g/kg respectively. In the present study, deficiencies were produced by using either casein or a pea (*Pisum sativum*) isolate as the protein source at 120 or 180 g/kg diet. Cys or Cys + Met supplementation was used to correct deficiencies in protein intake.

METHODS

Animals and experimental procedure

Male Wistar rats were obtained from IFFA Credo (l'Arbresle, France). They were housed in an air-conditioned room with a mean temperature of $21 \pm 1^\circ$, were weighed daily and the food intake calculated. Water and diets were offered *ad lib*.

Expt 1. Twenty-eight rats with an initial weight of 130–140 g, were randomized into three groups: one group of eight rats and two groups of ten rats each. They were fed for 28 d on one of the following three semi-purified diets (g/kg): casein 180, casein 120, casein 120 + 1.93 L-Cys (Table 1).

Table 1. *Composition of the diets (g/kg)*

Ingredients	Diet					
	Casein 180	Casein 120	Casein 120 + Cys	Pea* protein 180	Pea protein 120 + Cys	Pea protein 120 + Cys + Met
Casein (vitamin-free)†	180	120	120	—	—	—
Pea (800 g protein/kg)‡	0	—	—	219	157	157
Potato starch	400	436	434	381	428	428
Sucrose	315	339	339	308	329	329
Salt mixture§	40	40	40	40	40	40
Vitamin mixture	10	10	10	10	10	10
Agar agar	5	5	5	6	6	6
Peanut oil	50	50	50	30¶	36¶	36¶
L-Cysteine (Cys)	—	—	1.93	—	0.8	0.8
L-Methionine (Met)	—	—	—	—	—	0.6

* *Pisum sativum*

† USB - 12866; United States Biochemical Corporation, Ohio.

‡ Institut National de la Recherche Agronomique, Nantes.

§ USB - 21420; United States Biochemical Corporation. Contained (g/kg): CaCO₃ 210, CuSO₄ · 5H₂O 0.39, Fe₂(PO₄)₃ 14.70, MnSO₄ anhydrous 0.20, MgSO₄ anhydrous 90, KAl(SO₄)₂ 0.09, KCl 120, KH₂PO₄ 310, KI 0.05, NaCl 105, NaF 0.57, Ca₃(PO₄)₂ 149.

|| USB - 23431; United States Biochemical Corporation. Contained (g/kg): ascorbic acid 45.00, thiamin 1.00, riboflavin 1.00, pyridoxin 1.00, niacin 4.50, D-calcium pantothenate 3.00, inositol 5.00, choline 75.00, biotin 0.02, folic acid 0.09, vitamin B₁₂ 0.00135, menadione 2.25, α -tocopherol 5.00, calciferol (D₂) 0.0025, vitamin A acetate 0.31.

¶ Equivalent to 50 g total lipid/kg as the pea isolate contained 90 g lipid/kg.

Expt 2. Twenty-four rats with an initial weight of 110–120 g, were randomized into three groups of eight rats each. They were fed for 24 d on one of the following three semi-purified diets (g/kg): pea protein 180; pea protein 120 + 0.8 L-Cys; pea protein 120 + 0.8 L-Cys + 0.6 L-Met (Table 1).

At the end of the experimental period (Expts 1 and 2) the animals were killed by decapitation at 09.00 hours and blood and liver were very rapidly collected. The time between animal removal from the cages and blood collection was less than 30 s; an additional 30 s elapsed before washing excised liver in ice-cold buffer. A portion of blood was immediately used for glutathione analysis, the remainder was allowed to clot and the serum was stored at -23°. The liver was weighed and a portion used immediately for glutathione and protein analyses, the remainder was frozen in liquid nitrogen and stored at -80° for subsequent analysis. This method of killing and sampling minimized possible effects on the measured indices. GSH content, which is probably the most labile compound studied, was apparently not affected since the reduced form represented more than 97 % of total glutathione.

Analytical procedure

Hormone assays. Serum T₄ was determined by the competitive-binding assay of Murphy & Jachan (1965) adapted by Vigouroux (1972); serum T₃ was determined by the specific double-antibody radioimmunoassay of Chopra *et al.* (1972) adapted by Jordan *et al.* (1980).

Liver enzyme activities. Glucose-6-phosphate dehydrogenase (*EC* 1.1.1.49; *G6P-DH*) and phosphogluconate dehydrogenase (decarboxylating) (*EC* 1.1.1.44; *PG-DH*) were assayed according to Löhner & Waller (1965) and King (1965).

Table 2. *Weight increase, food intake, protein content of liver and serum in rats fed on the six experimental diets*
(Mean values with their standard errors)

Diet†	No. of rats	Cysteine (Cys) (g/kg dry diet)	Methionine (Met) (g/kg dry diet)	Wt increase (g/d)		Food intake (g/d)		Liver (mg/g wet wt)		Serum (g/l)		
				Mean	SE	Mean	SE	Mean	SE	Mean	SE	
Expt 1												
Casein 180	8	0.54	4.50	5.8 ^a	0.2	21.7 ^a	1.6	143 ^a	4	56.0 ^a	0.8	
Casein 120	10	0.36	3.10	2.8 ^b	0.1	18.6 ^a	1.2	122 ^b	3	54.5 ^a	0.5	
Casein 120+1.93 L-Cys	10	2.60	3.10	3.3 ^c	0.2	19.1 ^a	0.7	127 ^b	2	57.0 ^a	0.5	
Expt 2												
Pea (<i>Pisum sativum</i>) protein 180	8	2.60	1.80	4.3 ^a	0.1	21.5 ^a	1.5	150 ^a	3	51.5 ^a	0.7	
Pea protein 120+0.8 L-Cys	8	2.60	1.20	4.3 ^a	0.2	20.5 ^a	1.3	114 ^b	3	55.0 ^b	0.5	
Pea protein 120+0.8 L-Cys+0.6 L-Met	8	2.60	1.80	5.7 ^b	0.3	20.4 ^a	1.5	111 ^b	3	53.0 ^{ab}	0.8	

^{a, b, c} For each experiment, and within columns, mean values with different superscript letters were significantly different (Student's *t* test): *P* < 0.05.

† For details of diets, see Table 1.

Table 3. Effects of diets containing 180 g casein or 120 g casein/kg with or without cysteine (Cys) supplementation
(Mean values with their standard errors)

Diet*	Reduced glutathione				Serum thyroid hormones				Liver enzyme activities			
	Blood (mmol/l)		Liver (μ mol/g wet wt)		Thyroxine (ng/ml)		Triiodothyronine (ng/ml)		G6P-DH (IU†/g protein)		PG-DH (IU†/g protein)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Casein 180	1.30 ^a	0.04	6.70 ^a	0.35	45.50 ^a	3.50	0.83 ^a	0.05	60 ^a	6	65 ^a	4
Casein 120	1.23 ^a	0.04	2.54 ^b	0.15	45.90 ^a	1.70	0.30 ^b	0.03	39 ^b	3	51 ^b	3
Casein 120 + 1.93 L-Cys	0.92 ^b	0.02	4.29 ^c	0.63	42.70 ^a	1.20	0.49 ^c	0.04	34 ^b	4	46 ^b	3

^{a, b, c} Within columns, mean values with different superscript letters were significantly different (Student's *t* test): $P < 0.05$.

G6P-DH, glucose-6-phosphate dehydrogenase (EC 1.1.1.49); PG-DH, phosphogluconate dehydrogenase (decarboxylating) (EC 1.1.1.44).

* For details of diets, see Table 1.

† A unit of enzyme is defined as the amount of enzyme which reduces 1 μ mol NADP/min at 25°.

Table 4. Effects of diets containing 180 g pea (Pisum sativum) protein/kg or 120 g pea protein/kg supplemented with L-cysteine (Cys) or L-Cys and L-methionine (Met)
(Mean values with their standard errors)

Diet*	Reduced glutathione				Serum thyroid hormones				Liver enzyme activities			
	Blood (mmol/l)		Liver (μ mol/g wet wt)		Thyroxine (ng/ml)		Triiodothyronine (ng/ml)		G6P-DH (IU†/g protein)		PG-DH (IU†/g protein)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Pea protein 180	1.28 ^{ab}	0.04	7.50 ^a	0.28	53.1 ^a	2.3	0.75 ^a	0.04	32 ^a	4	38 ^a	1
Pea protein 120 + 0.8 L-Cys	1.21 ^a	0.04	2.11 ^b	0.11	43.9 ^b	1.8	0.40 ^b	0.01	61 ^b	7	73 ^b	6
Pea protein 120 + 0.8 L-Cys + 0.6 L-Met	1.33 ^b	0.01	1.39 ^c	0.06	36.6 ^c	1.2	0.33 ^c	0.02	48 ^b	5	67 ^b	6

^{a, b, c} Within columns, mean values with different superscript letters were significantly different (Student's *t* test): $P < 0.05$.

G6P-DH, glucose-6-phosphate dehydrogenase (EC 1.1.1.49); PG-DH, phosphogluconate dehydrogenase (decarboxylating) (EC 1.1.1.44).

* For details of diets, see Table 1.

† A unit of enzyme is defined as the amount of enzyme which reduces 1 μ mol NADP/min at 25°.

Protein assays. Serum and liver proteins were measured according to Bradford (1976) using the Bio-Rad protein assay (Bio-Rad laboratories, Munich, West Germany).

GSH assay. Enzymic analysis was performed according to Akerboom & Sies (1981).

Statistical analysis

Results were compared using Student's *t* test. Differences in mean values were considered significant at $P < 0.05$.

RESULTS

Expt 1

Effects of the diet containing 120 g casein/kg. As can be seen from Tables 2 and 3, reducing protein intake from 180 to 120 decreased body-weight gain and changed some liver and blood chemical indices. There were decreases in liver proteins, in liver enzyme activities of the pentose phosphate cycle, and in liver GSH content (whereas blood GSH was not affected). The serum level of T_4 was unchanged whereas that of T_3 was strongly decreased.

Effects of a diet containing 120 g casein/kg supplemented with 1.93 g L-Cys/kg. Compared with animals fed on the unsupplemented 120 g casein/kg diet, there was a higher body-weight gain, increased liver GSH content and serum level of T_3 . However none of these variables reached the values measured in rats fed on a 180 g casein/kg diet. The liver proteins and the liver enzyme activities of the pentose phosphate cycle remained at a low level.

Expt 2

Effects of a diet containing 120 g pea protein/kg supplemented with 0.8 g L-Cys. Tables 2 and 4 show that, when compared with 180 g pea protein/kg, this diet did not induce a change in body-weight gain but produced a decrease in liver GSH content (blood GSH was not affected) and in serum levels of T_4 and T_3 . There was a marked decrease in liver proteins and an unexpected increase in the activities of the pentose phosphate cycle liver enzymes.

Effects of a diet containing 120 g pea protein/kg supplemented with 0.8 g L-Cys and 0.6 g L-Met. With supplementation there was a higher decrease in the liver GSH content and the serum levels of both T_4 and T_3 . The activities of the pentose phosphate cycle liver enzymes remained at a high level. The body-weight gain was increased compared with that of rats fed on either a 180 g pea protein/kg diet or a 120 g pea protein/kg diet supplemented with 0.8 g L-Cys/kg only.

DISCUSSION

In the present study liver proteins were a better index of nutritional status than serum proteins which remained unchanged following reduction in dietary casein. It was also observed that when the liver protein concentration decreased, liver GSH content decreased, but it is not known if these changes were causally related. The 120 g casein/kg diet induced low levels of liver GSH because of Cys deficiency. This defect can be corrected either by Cys supplementation (120 g casein + 1.9 g L-Cys/kg) or by Cys resulting from the metabolism of part of the large amount of Met contained in the 180 g casein/kg diet. It is known that when L-Met increases in the diet, the amount of L-Cys produced from L-Met inevitably increases (Tateishi *et al.* 1981). Cho *et al.* (1984), using amino acid mixtures with levels of Cys and Met similar to those supplied in the diets used in the present experiments, observed similar changes in the liver GSH content. Normal levels of liver GSH were observed with the 180 g pea protein/kg diet. Reduction of the pea protein from 180 to 120 g/kg reduced

the level of liver GSH even with Cys or with Cys + Met supplementation. Thus other factors are probably implicated in the determination of the GSH levels. These factors may be related to the specific amino acid content of untreated pea protein, or the drastic technology applied in the preparation of the protein which could have reduced the availability of some amino acids, or both. These factors might interfere with Cys and Met levels and thus GSH metabolism.

The GSH level of blood, which essentially reflects the GSH level in erythrocytes (Akerboom & Sies, 1981), was not affected by the different diets used. This finding is in agreement with that of Cho *et al.* (1984).

The effects of a reduced protein intake on serum thyroid hormone levels were not clear (Brasel, 1980). Studies on rats fed on a low-protein diet have shown either an increase (Ingbar & Galton, 1975; Okamura *et al.* 1981) or a decrease (Yousef & Johnson 1968; Schussler & Orlando, 1978; Hasting & Zeman, 1979) in these levels.

The present results show that when the protein contained a satisfactory amino acid balance (i.e. casein), the reduction of protein intake (120 g/kg diet) did not affect the serum T_4 level. On the contrary, when the protein supplied was the pea protein the reduction in intake produced a decrease in T_4 even with L-Cys or L-Cys + L-Met supplementation. This decrease cannot be attributed to an increased metabolization of T_4 since the main metabolic pathway is a 5'-monodeiodination (Oppenheimer *et al.* 1970) using a monodeiodinase for which GSH is a cofactor (Visser *et al.* 1976; Chopra, 1978; Imai *et al.* 1980). However, it may be attributed to a decreased T_4 production resulting from a decreased hormonal synthesis by the gland and not from an inadequate supply of specific amino acids since the levels of phenylalanine (Phe) and tyrosine (Tyr) are similar in casein and in pea protein (44 g Phe and 56 g Tyr/kg casein; 51 g Phe and 35 g Tyr/kg pea protein).

In the rat the majority of T_3 results from the peripheral deiodination of T_4 (Abrams & Larsen, 1973). The present results show that when diets which affect liver GSH content are used there is a corresponding decrease in T_3 serum levels. Therefore the level of liver GSH could be implicated in the decrease in serum T_3 level reported in human protein-energy malnutrition and attributed, at least in part, to a decreased deiodination (Ingenbleek & Beckers, 1975). T_3 is known to be one of the factors which regulate the activities of the pentose phosphate cycle enzymes (Mariash *et al.* 1980). The results obtained in the present study show that the effect of T_3 on these enzymes is modulated by the quantity and quality of the dietary protein. In Expt 1 the reduction from 180 g to 120 g casein/kg diet induced a decreased T_3 serum level and decreased enzyme activities. When the 120 g casein/kg diet was supplemented with Cys there was a small but significant increase in serum T_3 but no change in enzyme activities. The results obtained in Expt 2 using pea protein are more complex and less easy to explain. The enzyme activities measured in rats receiving the 180 g pea protein/kg diet were reduced when compared with that of rats receiving the 180 g casein/kg diet. This could be due to an amino acid imbalance in the pea-protein preparation. The reason for the increased enzyme activities observed when 120 g pea protein/kg diet was supplemented with either Cys or Cys + Met, however, is not obvious.

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