Protein synthesis *in vivo* in rats fed on lipid-rich liquid diets

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Changes in tissue composition and protein synthesis ratio were studied in the major tissues of the body in young rats fed on lipid-rich, isonitrogenous purified liquid diets, a convenient method for inducing voluntary overfeeding under controlled nutritional conditions. Overfed rats showed faster growth induced by the energy excess. Analysis of tissue composition (protein, DNA and RNA contents) revealed that growth was due mainly to tissue hyperplasia in which protein and DNA contents increased in parallel. Fractional protein synthesis ratio measured *in vivo* by the flooding-dose method of phenylalanine showed a marked increase in all tissues. This change could be attributed to an increase in the ribosomal activity for protein synthesis in most tissues. Therefore, our results indicate that addition of a supplementary energy source (as lipids) to a well-balanced diet improves growth and protein synthesis in growing rats.

Overfeeding: Tissue composition: Protein synthesis

It is well known that the addition of a supplementary source of non-protein energy to an adequate diet improves nitrogen balance (Munro, 1951, 1964, 1978; Reeds *et al.* 1981). However, it still remains a challenge to define the long-term control mechanisms by which the energy excess affects N economy and particularly protein metabolism. To our knowledge, few studies have been done on changes in tissue growth patterns and protein turnover in overfeeding conditions. Indeed, most investigations in this area have been carried out using energy- and/or protein-restrictive conditions. These works have largely contributed to the advance in our understanding of the physiological and biochemical mechanisms that control changes in tissue protein mass (Waterlow *et al.* 1978; Reeds *et al.* 1980, 1981; Waterlow & Jackson, 1981; McNurlan *et al.* 1982; Kinney & Elwyn, 1983; Waterlow, 1986). Nevertheless, relationships between dietary energy excess and protein turnover have been poorly explored (Glick *et al.* 1982; Kita *et al.* 1989) despite the great interest that has been expressed in understanding the biochemical basis behind the plastic growth of animal tissues.

A major factor in nutritional studies is exact control of the nutrient intake of the animals. In a recent paper (Estornell *et al.* 1994) we have described an original overfeeding technique using lipid-rich, isonitrogenous, purified liquid diets. This accurate and easy method allows overfeeding of the animals with an excessive amount of lipid without changes in the quantitative and qualitative intake of the other nutrients (protein, carbohydrates, vitamins and minerals). Rats overfed in this way showed metabolic adaptive changes related to improved N metabolism. Similar alterations have been reported by us in previous papers using other models of overfeeding (Barber *et al.* 1985, 1987). However, it was not known whether changes in N metabolism were accompanied by changes in protein turnover.

In the present work, emphasis has been placed on studying the effects of the lipid–energy

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overfeeding on growth patterns, nucleic acid concentration and changes in protein synthesis in the major tissues of the body in growing rats. As protein turnover is strongly influenced by the nutrient content of the diet (Munro, 1964; Waterlow *et al.* 1978; Waterlow & Jackson, 1981; Reeds & James, 1983; Jansen & Hunsaker, 1986) we have taken special care to control diet composition and its intake by the animals to avoid the interference of other nutritional modifications affecting quantity or quality of diet components as previously described (Estornell *et al.* 1994).

EXPERIMENTAL

Animals and diets

Male Wistar rats (Panlab S.L., Barcelona, Spain) of 160–180 g body weight were placed in individual cages under controlled conditions of light (12 h light–12 h dark, lights on 08.00 hours) and temperature ($22 \pm 1°$). Control diet (diet C) was formulated to meet the National Research Council (1978) and American Institute of Nutrition (1977, 1980) recommendations. They were adapted to the liquid form as in our previous studies (Barber *et al.* 1990; Estornell *et al.* 1994). The composition (g/l) of diet C was: casein (vitamin-free) 52·0, DL-methionine 0·8, dextrin 162·7, maize oil 13·0, AIN-76A vitamin mix 2·6, AIN-76 mineral mix 9·1, choline chloride 0·3, cellulose powder 10·0, xanthan gum 2·0, in distilled deionized water. The energy of diet C was 4·18 MJ/l. Two lipid-rich, isonitrogenous liquid diets (H diets) were obtained exclusively by increasing the lipid concentration from the same source: diet H1 (57·7 g maize oil/l, 5·86 MJ/l) and H2 (41·0 g maize oil/l, 5·23 MJ/l). The control group was fed on diet C for 21 d. The overfed group was fed on diet H1 for 11 d and on diet H2 for another 10 d (for more details, see Estornell *et al.* 1994). Vitamin (AIN-76A) mix, mineral (AIN-76) mix and casein (micropulverized, vitamin-free) were purchased from ICN Biomedicals, High Wycombe, Bucks. Other compounds used for the formulation of diets were obtained from Sigma Chemical Co. St Louis, MO, USA.

Protein synthesis

Protein synthesis was measured *in vivo* by the phenylalanine flooding-dose method (Garlick *et al.* 1980). In brief, each animal was lightly anaesthetized with diethyl ether (Sampson *et al.* 1984) and injected via the femoral vein (1·0 ml/100 g body weight) with L-[4-^{3}H]phenylalanine ($7{\cdot}4 \times 10^5 - 1{\cdot}1 \times 10^6$ MBq/mmol; Amersham, Bucks) combined with unlabelled phenylalanine to give 150 mM and 1·85 MBq/ml. After precisely 10 min, rats were killed by decapitation and selected organs and tissues were rapidly excised, weighed and frozen between two aluminium blocks precooled on liquid N_2 (McNurlan *et al.* 1982; Goldspink & Kelly, 1984). Experiments were performed between 10.00 and 12.00 hours to minimize diurnal variations. Frozen tissue samples were pulverized under liquid N_2 and extracted with $HClO_4$ (Garlick *et al.* 1980). Specific radioactivity of the precursor both in the free tissue pool and covalently bound in protein was measured in the supernatant and the pellet respectively. This involved the prior hydrolysis of the washed protein pellets in 6 M-HCl at 110° for 24 h and the enzymic conversion of phenylalanine into β-phenylethylamine for its extraction. Radioactivity was measured by liquid scintillation and the concentration of the amine by a fluorimetric method (Garlick *et al.* 1980). The fractional rate of protein synthesis K_S (% per 24 h, percentage of the protein mass synthesized/d in the tissue) was calculated from the following (McNurlan *et al.* 1979; Garlick *et al.* 1983):

$$K_S = \frac{S_B \times 100}{S_A \times t}$$

where S_A and S_B are the specific radioactivity of phenylalanine in the free tissue pool and protein respectively (dpm/nmol), and t is the incorporation time of the precursor in days (time from injection to animal death).

Other methods

Analysis of N content was done by Kjeldahl digestion and Nessler's reaction (Minari & Zilversmit, 1963) on samples of urine and faeces collected during the last 48 h of the dietary treatment. Protein was measured by the method of Lowry *et al.* (1951) using bovine serum albumin as standard. RNA content was measured in the acid extracts by u.v. absorption with a correction for peptides (Munro & Fleck, 1969). DNA was also extracted and evaluated by the indole method using calf thymus DNA as standard (Munro & Fleck, 1969).

Presentation of data

Data in the tables are presented as the mean value of each set of animals. Variability was estimated by the standard error of the mean (SEM). Data from control and overfed groups were subjected to one-way analysis of variance and significance tests were carried out at the 5% level.

RESULTS AND DISCUSSION

Table 1 shows the total nutritional treatment of control and overfed groups. Lipid intake in overfed animals increased 3·7-fold and they ingested 32% more energy in the 21 d dietary treatment than control animals. Diet volume intake was kept constant during the whole period (52·8 (SE 1·4) ml/24 h, n 12 in the control group and 53·1 (SE 1·0) ml/24 h, n 12 in the overfed group). Therefore, there were no further modifications in protein, carbohydrate, vitamin and mineral intakes. The excessive intake of energy and lipids promoted a higher development in body weight in the overfed growing rats (20%) when compared with controls as shown in Table 1. This difference could be due to either a higher development of the whole body or an individual response of some tissues (e.g. adipose tissue mass). Data from N balance (Table 1) seem to support the first statement with an improved utilization of dietary N in the overfed group, despite the fat gain (Estornell *et al.* 1994).

The response in the whole-body weight to the overfeeding was paralleled by the individual tissues, as shown in Table 2. In fact, liver, kidney, heart and skeletal muscles (*gastrocnemius* and *tibialis anterior*) increased their masses with minimal variations in the relative contribution of each one to the whole-body weight. Jejunal serosa showed an enlargement without changes in the small-intestine length, whereas the mucosa did not change. Tissue protein concentration (mg/g) did not change between groups (results not shown); therefore, the total protein content of each tissue increased in the overfed group. Percentages of change were similar to those of tissue weights. DNA content was evaluated as an index of the nuclear proliferation in the tissues (Goldspink & Kelly, 1984). Table 2 shows that this variable changed approximately according to tissue weight; however, heart, *tibialis* and jejunal serosa showed lower relative increments.

Tissue RNA content is commonly accepted as an estimate of the ribosomal content of the cells because more than 80% of the total RNA is contained in the ribosomes (Munro & Fleck, 1969; Waterlow *et al.* 1978). Table 3 shows that RNA content was higher in the overfed-group tissues, except in jejunal mucosa and serosa which did not change. The RNA:DNA ratio allowed us to evaluate whether there was a real increment in the ribosomal density due to a greater nuclear activity or not. As shown in Table 3, the RNA:DNA ratio remained constant in all tissues, except in liver. In this organ there was a notable increment in RNA content over the protein and DNA contents. Table 3 also

Table 1. *Dietary treatment and physiological variables of rats fed on a liquid control diet (4·18 MJ/l) for 21 d, or overfed on a lipid-rich liquid diet (5·86 MJ/l) for 11 d and on a second lipid-rich liquid diet (5·23 MJ/l) for the remaining 10 d**

(Mean values with their standard errors for twelve rats)

	Control		Overfed		Statistical significance of treatment effects: *P*
	Mean	SEM	Mean	SEM	
Dietary intakes					
Energy (MJ)	4·63	0·06	6·09	0·11	< 0·05
Protein (g)	58·4	0·8	57·9	0·8	NS
Lipid (g)	14·59	0·15	53·6	1·0	< 0·05
Carbohydrate (g)	184	2	184	3	NS
Body wt					
Initial (g)	170·9	1·2	171·4	1·9	NS
Final (g)	228	3	275	6	< 0·05
N balance† (mg N/24 h)	203	2	275	4	< 0·05

NS, not significant.

* For details of diets, see p. 510.

† Nitrogen balance was evaluated during the last 48 h of the dietary treatment.

shows the protein:DNA ratio. This ratio is a useful index of cell size, in spite of the limitation for liver and skeletal muscle because of the existence of polyploidy (Goldspink & Kelly, 1984). Our data show that the protein:DNA ratio remained unmodified in the overfed-animal tissues. Only heart developed a slight hypertrophy. From the results of the tissue composition reported in Tables 2 and 3 we can conclude that an excess of dietary energy as lipids, at the same level and quality of dietary protein, stimulated the growth of the animals and this was mainly characterized by tissue hyperplasia.

Net protein gain depends on two important processes: protein synthesis and protein breakdown. Both are independently regulated. The protein accretion can be due to an increment in protein synthesis, a reduction in protein breakdown or a parallel change in both processes resulting in an increment of tissue protein content (Waterlow & Jackson, 1981; Reeds & James, 1983). Because of the availability of a direct and accurate method for measuring protein synthesis *in vivo*, we studied the individual response of the major tissues of the body to the lipid overfeeding. Table 4 shows the fractional protein synthesis (K_S, percentage of the protein mass synthesized/d) in the different tissues. In all cases, K_S increased in overfed-rat tissues, which showed values about 30% higher than those found in control-rat tissues. When synthesis rates were calculated to take account of the tissue size, the total amount of protein being synthesized/d in each one ($K_S \times$ protein mass) increased more markedly (results not shown). The differences found in the total protein synthesis (mg/d) between overfed and control groups became greater than those in the corresponding variable.

The rate of protein synthesis depends on the number of ribosomes per cell and the activity of these ribosomes in the translational process; therefore, it is useful to examine these variables in parallel. An index of ribosomal capacity can be obtained from RNA:protein values and that of ribosomal activity from synthesis/unit RNA (Waterlow *et al.* 1978; Goldspink & Kelly, 1984). Table 4 shows that the increase in the fractional rate of synthesis observed in the overfed group could be attributed to an enhancement in the ribosomal activity or synthetic efficiency of ribosomes, whereas ribosomal capacity

Table 2. *Weight, and protein and DNA contents of organs and tissues from rats fed on a liquid control diet (4·18 MJ/l) for 21 d, or overfed on a lipid-rich liquid diet (5·86 MJ/l) for 11 d and on a second lipid-rich liquid diet (5·23 MJ/l) for the remaining 10 d**

(Mean values with their standard errors for six rats)

	Control						Overfed								
	Weight (g)		Protein content (mg)		DNA content (mg)		Weight (g)			Protein content (mg)			DNA content (mg)		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	*P*	Mean	SEM	*P*	Mean	SEM	*P*
Liver	9·9	0·3	1760	60	22·1	0·9	11·8	0·7	< 0·05	2250	90	< 0·05	28·3	1·7	< 0·05
Kidney (both)	1·45	0·02	258	6	4·08	0·09	2·02	0·06	< 0·05	342	10	< 0·05	5·4	0·4	< 0·05
Heart	0·75	0·02	140	3	1·08	0·04	1·00	0·02	< 0·05	177	4	< 0·05	1·15	0·07	NS
Skeletal muscles															
gastrocnemius	1·40	0·06	283	13	0·87	0·04	1·70	0·03	< 0·05	333	8	< 0·05	1·04	0·04	< 0·05
tibialis	0·443	0·014	93	2	0·308	0·004	0·578	0·012	< 0·05	126	5	< 0·05	0·449	0·009	NS
Jejunum															
mucosa (mg/cm)	42	2	5·9	0·4	0·184	0·006	45	2	NS	6·5	0·2	NS	0·218	0·013	< 0·05
serosa (mg/cm)	40	2	6·9	0·4	0·160	0·011	48·9	1·8	< 0·05	8·1	0·3	< 0·05	0·177	0·004	NS

NS, not significant.

* For details of diets and procedures, see pp. 510–511.

Table 3. *RNA content and RNA : DNA and protein : DNA ratios in organs and tissues from rats fed on a liquid control diet (4·18 MJ/l) for 21 d, or overfed on a lipid-rich liquid diet (5·86 MJ/l) for 11 d and on a second lipid-rich liquid diet (5·23 MJ/l) for the remaining 10 d**

(Mean values with their standard errors for six rats)

	Control						Overfed								
	RNA content (mg)		RNA:DNA ratio		Protein:DNA ratio		RNA content (mg)			RNA:DNA ratio			Protein:DNA ratio		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	*P*	Mean	SEM	*P*	Mean	SEM	*P*
Liver	60·3	1·8	2·74	0·09	80	3	88	5	< 0·05	3·10	0·07	< 0·05	80	3	NS
Kidney (both)	4·45	0·07	1·09	0·02	63	2	6·4	0·2	< 0·05	1·22	0·08	NS	64	4	NS
Heart	1·58	0·06	1·48	0·08	130	4	1·88	0·06	< 0·05	1·65	0·09	NS	156	8	< 0·05
Skeletal muscles															
gastrocnemius	2·06	0·13	2·37	0·10	326	5	2·53	0·13	< 0·05	2·44	0·10	NS	322	10	NS
tibialis	0·75	0·03	2·44	0·08	303	5	1·03	0·05	< 0·05	2·30	0·10	NS	280	9	NS
Jejunum															
mucosa†	0·34	0·03	1·87	0·15	32·1	1·9	0·40	0·02	NS	1·87	0·08	NS	30·2	1·3	NS
serosa†	0·206	0·018	1·29	0·07	43·2	1·7	0·244	0·016	NS	1·37	0·08	NS	45·6	1·2	NS

NS, not significant.

* For details of diets and procedures, see pp. 510–511.

† RNA content expressed as mg/cm.

Table 4. *Fractional protein synthesis, ribosomal activity and ribosomal capacity in organs and tissues from rats fed on a control liquid diet (4·18 MJ/l) for 21 d, or overfed on a lipid-rich liquid diet (5·86 MJ/l) for 11 d and on a second lipid-rich liquid diet (5·23 MJ/l) for the remaining 10 d**

(Mean values with their standard errors for six rats)

	Control						Overfed								
	Fractional synthesis K_S (% per 24 h)		Ribosomal activity (mg protein synthesized/ g RNA × 24 h)		Ribosomal capacity (mg RNA/ g protein)		Fractional synthesis K_S (% per 24 h)			Ribosomal activity (mg protein synthesized/ g RNA × 24 h)			Ribosomal capacity (mg RNA/ g protein)		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	*P*	Mean	SEM	*P*	Mean	SEM	*P*
Liver	78	2	22·7	0·8	34·2	0·9	96	2	< 0·05	24·6	0·8	NS	38·9	1·1	< 0·05
Kidney (both)	73·1	1·3	42·3	1·1	17·3	0·4	96	3	< 0·05	51·6	1·4	< 0·05	18·7	0·3	NS
Heart	16·3	0·8	14·6	1·5	11·4	0·6	22·2	0·8	< 0·05	20·9	0·7	< 0·05	10·6	0·21	NS
Skeletal muscles															
gastrocnemius	14·7	0·6	20·2	0·6	7·3	0·3	17·4	0·8	< 0·05	23·0	1·1	< 0·05	7·6	0·4	NS
tibialis	9·0	0·4	11·3	0·8	8·1	0·2	13·6	0·7	< 0·05	16·6	1·1	< 0·05	8·2	0·2	NS
Jejunum															
mucosa†	125	6	21·7	1·0	57·9	1·7	166	4	< 0·05	27·1	1·3	< 0·05	62	2	NS
serosa†	75·0	1·6	25·6	1·6	29·7	1·3	91·2	1·8	< 0·05	30·5	1·4	< 0·05	30·2	1·6	NS

NS, not significant.

* For details of diets and procedures, see pp. 510–511.

† Ribosomal activity expressed as mg protein synthesized/cm × g RNA × 24 h.

remained unchanged, except in liver. The increment in the ribosomal capacity shown by the liver was a direct consequence of the marked increase in its RNA content (Table 3). The different response of this organ in the context of the whole body probably needs further studies to confirm it. These results for protein synthetic activity in the different tissues analysed seem to show that overfeeding under our experimental conditions, stimulated the protein synthesis process by an enhancement of the ribosomal efficiency.

Protein breakdown is difficult to measure *in vivo* (McNurlan *et al.* 1979; Odedra *et al.* 1983; Reeds & James, 1983; Muramatsu *et al.* 1990). However, since growth arises from an imbalance in the rates of synthesis and degradation, protein breakdown can be calculated by subtraction of fractional rates of synthesis and growth (Odedra *et al.* 1983; Goldspink & Kelly, 1984). Growth rate (net accumulation of protein in the tissue/d) can be estimated from the relative body-weight increment (% per 24 h) and the protein content of the tissue (Odedra *et al.* 1983; Muramatsu & Okumura, 1985; Kita *et al.* 1989). In this way, we can assume that fractional protein growth in each tissue paralleled the whole-body growth. From this it is apparent that changes in protein synthesis largely exceeded those in net protein gain and therefore protein breakdown was also increased in overfed rats (calculations not shown). Hence, under our experimental conditions, overfeeding seems to induce a faster protein turnover rate.

Assuming that the rates of protein synthesis in *gastrocnemius* and *tibialis* muscles are representative of all skeletal muscle, the values obtained in the seven tissues measured account for at least 80% of the total protein synthesis in the whole animal (McNurlan *et al.* 1982; Goldspink & Kelly, 1984). Therefore, we can conclude that availability of a supplementary source of dietary energy as lipid, at an adequate level and quality of protein, promotes an important increase in protein turnover rate with a net increase in protein gain. A similar change (protein accretion with increased synthesis and breakdown) has been described during compensatory growth after a period of malnutrition and during functional overloading of tissues (Waterlow *et al.* 1978; Reeds & James, 1983; Waterlow, 1986). Since the energy cost of protein deposition is very high in this situation (Kinney & Elwyn, 1983; Waterlow, 1986) we suggest that it could contribute to dissipation of the excess energy ingested with a concomitant diminution in the metabolic efficiency of food (Waterlow, 1986; Kita *et al.* 1989).

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