Low-protein diet prevents tissue lipoprotein lipase activity increase in growing rats

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The time course of changes in tissue lipolytic activities was studied in young rats during the consumption of a low-protein diet containing 50 g protein/kg (40 g wheat gluten +10 g casein/kg) for 28 d followed by balanced refeeding with 200 g protein/kg (160 g wheat gluten +40 g casein/kg) for 28 d. Lipoprotein lipase (LPL) activities were compared with the values of a control group fed a balanced diet containing 200 g protein/kg for 56 d. At the end of protein malnutrition period, the epididymal fat tissue LPL activity represented 36 %, and that of heart and gastrocnemius was 44 %, of those of the control group. These differences were accompanied by lower serum- and VLDL-triacylglycerols (TAG), respectively 47·6 % and 31 % of the control group values, probably resulting from reduced synthesis of VLDL-apolipoproteins (29 % of control group values), concomitant with liver lipid accumulation (4·8-fold) and little lipid storage in epididymal fat tissue. At day 2 of refeeding, there was no significant difference in liver and epididymal fat tissue LPL activities between experimental and control rats. At the end of the refeeding period, LPL activity of epididymal fat and liver lipolytic activity had increased and became similar to control group values. The consumption of a low-protein diet prevented the increase in extrahepatic LPL activities as observed in the control group. The alterations in LPL activity suggest that a low-protein diet limits lipid storage in adipose tissue due to reduced serum VLDL-TAG availability.

Lipoprotein lipase: Protein malnutrition: Balanced refeeding: Rat

Hepatic triacylglycerol (TAG) lipase (TAG acyl hydrolase) and lipoprotein lipase (TAG-protein acyl hydrolase) catalyse the hydrolysis of plasma lipoprotein TAG. They play key roles in vascular lipoprotein metabolism through an efficient transfer of energy in the form of lipids from the sites of synthesis to those of storage or utilisation. The major tissues in which lipoprotein lipase (LPL) activity has been found include adipose tissue, heart, muscles and several other tissues (Soteriou & Cryer, 1993). LPL, present at the luminal surface of the capillary endothelium, is synthesised predominantly by parenchymal cells and requires, for maximal activity, apolipoprotein (Apo) C-II, an activating cofactor which is found in plasma associated mainly with chylomicrons, VLDL and HDL (Goldberg *et al.* 1990; Ikeda *et al.* 1989). The substrates for LPL are chylomicrons and VLDL which, by hydrolysis, turn into smaller remnants and are rapidly cleared from the blood stream. Although LPL is considered an essentially extra-hepatic enzyme, substantial LPL activity can also be found in the liver. In contrast to LPL, hepatic lipase (HL), which is almost exclusive to hepatic sinusoids, does not require ApoC-II as activator (Olivecrona & Bengtsson-Olivecrona, 1990). HL, i.e. TAG hydrolase of hepatic extract differed from LPL in that it was insensitive to Apo cofactor and was not inhibited by preincubation with a high concentration of NaCl or protamine sulfate. Although the physiological function of HL has not been clearly defined, there is increasing evidence that it also acts as a phospholipase (Kuusi *et al.* 1979*a*). HL and LPL localisations facilitate the cellular uptake of lipolytic products (free fatty acids and monoacylglycerols) (Brunzell, 1989; Eckel, 1989) available to the adjacent cells for oxidation or resynthesis into TAG for storage.

HL and LPL have been largely investigated during

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starvation followed by refeeding, and during the consumption of a low-energy diet, but not during protein malnutrition followed by balanced refeeding. Protein malnutrition involves a low level of circulating TAG, due to a significant VLDL diminution in young rats (Flores *et al.* 1970*a*) and infants (Truswell *et al.* 1969; Flores *et al.* 1970*b*; Waterlow, 1975). The origin of this low plasma VLDL level is a decrease in VLDL-Apo synthesis in both kwashiorkor patients (Seakins & Waterlow, 1972) and protein-depleted rats (Meghelli-Bouchenak *et al.* 1987, 1989*a,b*). Moreover, in protein-depleted rats, Agbedana (1980) showed that LPL activity is significantly reduced in adipose tissue, whereas there are no significant changes in heart and liver lipolytic activities. In a previous study, we have shown that total plasma lipolytic activity, which represents HL and LPL activities, remains low and stable in rats throughout the period of protein malnutrition (Lamri *et al.* 1995). We have also observed that VLDL composition varies significantly with the type of protein malnutrition (50 g wheat gluten or 20 g casein/kg). These modifications could be attributable to the various low levels of plasma VLDL-ApoC-II in rats fed protein-depleted diets, which may modify their activating effect on LPL activity.

The aim of the present study was to determine whether liver total lipolytic activity, namely HL and LPL activity in epididymal fat, gastrocnemius muscle and heart, were important in determining fatty acid availability to tissues during the consumption of a low-protein diet (40 g gluten +10 g casein/kg) followed by balanced refeeding in young rats. Moreover, the amount of TAG in liver and serum VLDL were investigated in order to see the organ capacity for release of free fatty acids from this substrate. We have chosen this protein composition because in numerous developing countries, particularly in Algeria, proteins originate mainly from cereals and to a lesser extent from dairy products. Since gluten, the main cereal protein, has a low lysine level, casein was added to the gluten diets, thus increasing their lysine level and therefore remedying their deficiency.

Material and methods

Animals and diets

Male Wistar rats (*n* 72) at weaning (Iffa Credo, l'Arbresle, Lyon, France) weighing 40 (SE 5) g at the beginning of the experiment were allowed free access to a balanced diet containing 200 g protein (160 g gluten +40 g casein/kg) for 5 d. After this adaptation period, they weighed 60 (SE 5) g and were then randomised into equal groups. A control group was fed the same balanced diet for 56 d. The experimental group was fed a low-protein diet containing 50 g protein (40 g gluten +10 g casein/kg) for 28 d (protein malnutrition period) and then the balanced diet for 28 d (balanced refeeding period). Table 1 shows the weight and energetic compositions of both diets which were isoenergetic. In the low-protein diet, the loss of energy from protein was compensated for by corn starch.

Rats were kept in wire-bottomed cages under an automatic lighting schedule (lights on 07.00 to 19.00 hours), at constant temperature (24°C) and humidity (60

Table 1. Diet composition*

Ingredients	40 g Casein+160 g gluten/kg (g/kg diet)	10 g Casein+40 g gluten/kg (g/kg diet)
Milk casein†	40	10
Wheat gluten‡	160	40
Corn starch‡	512	638
Sucrose§	98	122
Sunflower oil§	80	80
Cellulose‡	50	50
Mineral mix‖	40	40
Vitamin mix¶	20	20

* Diets were isoenergetic (1657 kJ/kg) and were given in powdered form.
† Merck, Darmstadt, Germany.
‡ UAR; Villemoisson 91360, Epinay S/Orge, France.
§ Commercial Products, Oran, Algeria.
‖ UAR 205 B; Villemoisson, Epinay S/Orge, France. Mineral mix provided (g/ kg diet): Ca 4, K 2·4, Na 1·6, Mg 0·4, Fe 0·12, Mn 0·032, Cu 0·005, Zn 0·018, Co 0·00004, I 0·00002.
¶ UAR 200; Villemoisson, Epinay S/Orge, France. Vitamin mix provided (mg/ kg diet): thiamin 40, riboflavin 30, nicotinic acid 140, pyridoxine 20, pyridoxal 300, cyanocobalamin 0·1, ascorbic acid 1600, α-tocopherol 340, menadione 80, calcium panthotenate 200, choline 2720, pteroylmonoglutaric acid 10, *p*-aminobenzoic acid 100, biotin 0·6, retinol 12, cholecalciferol 0·125.

%). Diets and water were freely available. We followed the general guidelines of the Council of European Communities (1986) for the care and use of laboratory animals. The body weight and food intake were measured daily from days 1 to 7 of protein malnutrition (period 1) and balanced refeeding (period 3) and from days 22 to 28 of protein malnutrition (period 2) and refeeding (period 4).

Substrate preparation for the lipolytic activities

The substrate emulsion was prepared according to the method of Okabe *et al.* (1984): 250 mg unlabelled triolein (Prolabo, Paris, France) were mixed with 0·185 MBq glycerol tri(9-10(*n*)-^{3}H)oleate (NET 431 NEN, Boston, MA, USA). After organic solvent removal under N_2 stream, the mixture was sonicated with 4 g bovine serum albumin fraction V/l (Merck, Darmstadt, Germany), 2 ml Triton X100 (10 ml/l) and 20 ml 0·2 M-Tris–HCl buffer (pH 8·6).

Tissue preparation for lipoprotein lipase activity determination

At days 2, 14 and 28 of protein malnutrition and days 2, 14 and 28 of balanced refeeding, after an overnight fast, six rats from each group were bled from the abdominal aorta under anaesthesia (60 mg sodium pentobarbital/kg body weight). Epididymal fat, heart, gastrocnemius muscle and liver were quickly excised and samples for enzyme activity analysis obtained by extraction with acetone and diethyl ethyl; these samples, which account for both the extracellular and the inactive intracellular LPL compartments, were used as the defatted preparation for measuring total lipolytic activity, according to the method of Hamosh *et al.* (1970): 200 mg of each tissue were washed in cold 0·15 M-NaCl to remove traces of blood and were homogenised in 8 ml cold acetone. The insoluble tissue constituents in acetone were collected after filtration through Whatman paper no. 5 on a Buchner funnel. The tissue residue was washed three times with

cold diethyl ether. The defatted preparation was dried, scraped off the filter paper and solubilised in cold 0·2 M-Tris–HCl buffer (pH 8·6) containing 1000 U heparin/l. The residue was removed from each extract by centrifugation of 4°C for 20 min. The supernatant was used as the sample for enzyme activity analysis.

Enzyme assay

Lipolytic activities in samples, prepared as described earlier, were measured by the release of labelled oleic acid from 9-10 $(n)^3$H-triolein with bovine serum albumin as fatty acid acceptor. The sample (5 ml) was incubated with 0·5 ml substrate emulsion containing heat-inactivated pooled serum (50 ml/l) as ApoC-II source. The serum was prepared from rat blood inactivated at 56°C for 45 min. After 1 h at 37°C, the incubation was stopped by the addition of 4 ml methanol. The heat-inactivated pooled serum used in the assay contained a negligible amount of TAG (0·09 mg) compared with triolein (6·5 mg) and its contribution (1 %) was a negligible dilution source of the specific activity. ApoC-II from the plasma (0·5 ml) in the assay was sufficient for maximal activating effect on lipolytic activity.

Lipid extraction

Lipid extraction on incubation was performed by the method of Folch *et al.* (1957). Total lipids were separated by TLC with hexane–diethyl ether–acetic acid (70:30:1, by vol.) as eluant. The different lipids were revealed by plate exposure to I vapours and directly scraped into scintillation vials containing 10 ml Ready solv HP (Beckman, Palo Alto, CA, USA) as scintillant. The samples were counted in Beckman LS 7500 scintillation counter (Beckman Instruments, Palo Alto, CA, USA). Total release of free fatty acids was calculated according to the method of Hills & Mukherjee (1988): (radioactivity (Bq) at origin/(radioactivity (Bq) at origin+radioactivity (Bq) in remainder))×TAG (μmol) in assay×3, and was expressed as μmol/h per g fresh tissue.

VLDL isolation

The technique used has been detailed in a previous paper (Meghelli-Bouchenak *et al.* 1991). Serum samples (2 ml) were overlaid with 4 ml 0·15 M-NaCl and 0·001 M-Na₂ EDTA (density 1·006 kg/l). After centrifugation at 15°C for 17 h at 108 000 *g* in a Beckman L8-55 Ultracentrifuge equipped with a 50 Ti rotor (Beckman Instruments), floating VLDL were isolated. VLDL fractions were subjected to partial lyophylisation, followed by rapid delipidation with cold diethyl ether at 4°C in order to avoid the precipitation of high molecular mass Apo.

Chemical analysis

The total lipids in the liver were extracted according to the method of Folch *et al.* (1957). An aliquot of liver total lipid, serum- and VLDL-TAG were determined by the enzymatic method using glycerol as standard (Boehringer, Mannheim,

Germany). Protein contents of VLDL were measured by the method of Lowry *et al.* (1951) using bovine serum albumin (Sigma, L'Isle d'Abeau, France) as standard.

Statistical analysis

Values are means with their standard errors. Statistical evaluation of the data was carried out using Statistica (version 4.1, Statsoft, Inc., 1994; Paris, France). Data were tested by two-way ANOVA and classification of the means was performed using Duncan's multiple range test (1955): (1) between both groups at each sampling time; (2) in the same group at different times during protein malnutrition and balanced refeeding. Linear regression analysis was used to determine the coefficients of correlation between some serum indices and liver steatosis. Statistical significance was accepted at $P < 0.05$.

Results

Body weight and food intake

After 28 d protein malnutrition, the body weights of the experimental rats was only 40 % of those of control rats (Fig. 1). Balanced refeeding promoted a rapid weight increase in the experimental group, but at day 28 of balanced refeeding, their weights were only 70 % of those of the control rats. The daily food intake per rat (Table 2) was similar, except at the end of malnutrition (period 2) and at the beginning of refeeding (period 3) when the values in the experimental group represented 50 % and 55 %
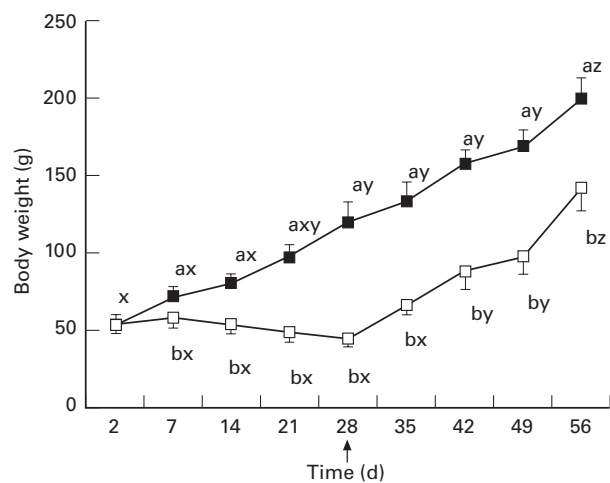
Fig. 1. Rat body weights during the consumption of the low-protein diet followed by refeeding the balanced diet. Values are means for six rats per group with their standard errors shown by vertical bars. The control group (-■-) was fed the balanced diet (160 g wheat gluten +40 g casein/kg) for 56 d. The experimental group (-□-) was fed the low-protein diet (40 g wheat gluten + 10 g casein/kg) for 28 d (protein malnutrition) then was refed the balanced diet for 28 d (balanced refeeding). ↑, Change of diet. For details of composition of diets see Table 1. [a,b]Mean values were significantly different from those of the control group (two-way ANOVA and Duncan's multiple range test): $P < 0.05$. [x,y,z]Mean values were significantly different within a group over time (two-way ANOVA and Duncan's multiple range test): $P < 0.05$.

Table 2. Food and energy intakes of rats during protein malnutrition followed by balanced refeeding*
(Mean values with their standard errors for six rats per group)

| | Protein malnutrition | | | | Balanced refeeding | | | |
| | Period 1 (days 1–7) | | Period 2 (days 22–28) | | Period 3 (days 1–7) | | Period 4 (days 22–28) | |
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Food intake (g/d per rat)								
Control group	5·44[x]	0·77	6·68[axy]	1·02	7·87[ay]	0·56	4·31[x]	0·94
Experimental group	4·61	0·89	3·37[b]	0·91	5·30[b]	0·43	4·34	0·79
Food intake (g/d per 100 g BW)								
Control group	9·91[x]	0·45	6·68[y]	0·55	6·56[by]	0·81	2·06[z]	0·36
Experimental group	9·58[x]	2·05	7·18[x]	2·64	9·57[ax]	0·42	3·17[y]	0·61
Energy intake (kJ/d per $W^{0.75}$)								
Control group	813·21[x]	40·66	628·90[y]	54·70	655·43[y]	44·50	236·58[z]	37·44
Experimental group	756·12[x]	170·20	572·62[x]	110·70	753·27[x]	37·21	327·25[y]	50·20

BW, body weight; $W^{0.75}$, metabolic body weight.
[a,b]Mean values within a column with unlike superscript letters were significantly different (two-way ANOVA and Duncan's multiple range test; $P < 0.05$).
[x,y,z]Mean values within a row with unlike superscript letters were significantly different (two-way ANOVA and Duncan's multiple range test; $P < 0.05$).
* The control group were fed the balanced diet (160 g wheat gluten + 40 g casein/kg) for 56 d. The experimental group were fed the low-protein diet (40 g wheat gluten + 10 g casein/kg) for 28 d (protein malnutrition) then refed the balanced diet for 28 d (balanced refeeding). For details of composition of diets see Table 1.

respectively of those of control rats. However, the metabolisable energy intake calculated factorially from food consumption and expressed as kJ/d per kg metabolic body weight ($W^{0.75}$) was similar in both groups at each period, but the values were only 30 % and 43 % in the control and experimental groups respectively at the end of the study (period 4) compared with the values obtained at the beginning of the experiment (period 1).

Relative weights of tissues

Changes in tissue weights are expressed as a percentage of body weight (Fig. 2). During protein malnutrition, gastrocnemius, heart and liver relative weights in protein-depleted rats were not significantly different from those of the control rats. In contrast, at day 14 of protein malnutrition, the relative weight of epididymal fat was significantly lower than that of the control group. At day 28 of protein malnutrition, it represented only 30 % of the control values. At day 2 of refeeding, only relative weight of the heart was increased and became significantly higher in the experimental group than that of the control group, whereas that of epididymal fat remained significantly lower.

Lipoprotein lipase activity of tissues

In the control group, the highest LPL activities per g fresh tissue were found in epididymal fat, whereas the lowest lipolytic values were obtained in the liver (Fig. 3). At day 2 of protein malnutrition, only liver lipolytic activity of the experimental group was significantly higher than that of the control group. At day 14 of protein malnutrition, LPL activities of epididymal fat and heart were significantly reduced. At the end of protein malnutrition, epididymal fat LPL activity was reduced to 33·4 µmol free fatty acids released/h per g tissue which represented only 30 % of the control value (102·4 µmol free fatty acids released/h per g). Gastrocnemius and heart LPL activities were less impaired, with 6·8 and 5·6 µmol free fatty acids released/h per g respectively which represented 44 % of control values. At day 2 of balanced refeeding, LPL activities were increased

and became similar in epididymal fat and even greater in the liver of the experimental group compared with those of control rats. However, in these tissues lipolytic activities decreased at day 14 of refeeding. At the beginning of refeeding, only heart and gastrocnemius lipolytic activities remained lower than control values. At the end of refeeding, LPL activities of epididymal fat and liver increased and became similar to control values. Gastrocnemius and heart LPL activities increased progressively during balanced refeeding; however, only heart LPL activity was still significantly lower in the experimental group.

Liver triacylglycerol content and serum VLDL-apolipoprotein and -triacylglycerol concentrations

At the beginning of the experiment (day 0), the hepatic TAG values were about 9·02 µmol/g liver. In the experimental group, a progressively greater liver TAG concentration was noted which was particularly high at day 28 of protein malnutrition when the values were 4·86-fold higher, whereas serum TAG amounts were 2·10-fold lower, than those of the control group (Table 3). Moreover, values for VLDL-TAG and Apo progressively decreased from day 2 to day 28 of protein malnutrition when the values were 3·21 and 3·37-fold lower in the experimental group than in the control group respectively. In the experimental group during protein malnutrition, there was a significant negative correlation between VLDL-Apo and liver TAG content (r −0·89, $P < 0.01$) on the one hand, and between VLDL- and liver TAG content (r −0·88, $P < 0.01$) on the other hand. During balanced refeeding, the same inverse relationship was observed between VLDL-Apo, VLDL-TAG content and hepatic steatosis (r −0·93, $P < 0.01$).

Discussion

In the present experiment, we chose not to use pair-fed controls for the depleted rats because the rats that consumed low-protein diets ate less and their body weights were lower, but on a body weight basis, the energy intake was the same. Therefore, we are essentially dealing with
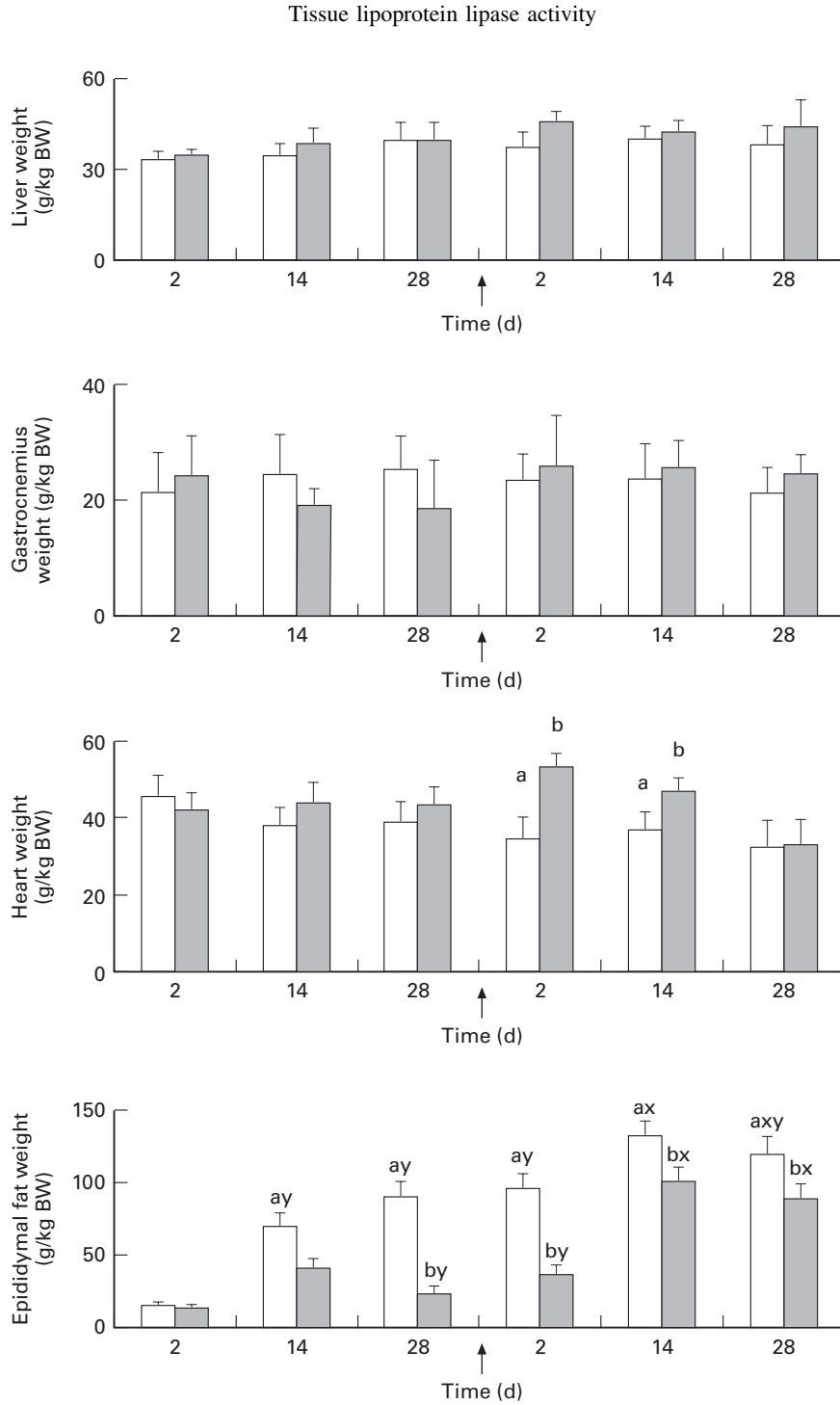
Fig. 2. Relative weights of liver, gastrocnemius, heart and epididymal fat (expressed as g tissue/kg body weight (BW). Values are means for six rats per group with their standard errors shown by vertical bars. The control group (□) was fed the balanced diet (160 g wheat gluten + 40 g casein/kg) for 56 d. The experimental group (▨) was fed the low-protein diet (40 g wheat gluten + 10 g casein/kg) for 28 d (protein malnutrition) then was refed the balanced diet for 28 d (balanced refeeding). ↑, Change of diet. For details of the composition of diets see Table 1. [a,b]Mean values were significantly different from those of the control group (two-way ANOVA and Duncan's multiple range test): $P < 0.05$. [x,y,z]Mean values were significantly different within a group over time (two-way ANOVA and Duncan's multiple range test): $P < 0.05$.

protein malnutrition. In addition, the two specific symptoms of protein malnutrition, i.e. liver steatosis and oedema, were observed with a similar experimental protocol in a previous study (Meghelli-Bouchenak *et al.* 1989b).

The protein-depleted diet (40 g gluten +10 g casein/kg) immediately stopped rat growth (Fig. 1), whereas the control rat weights were enhanced with age. It was therefore necessary to compare the experimental group
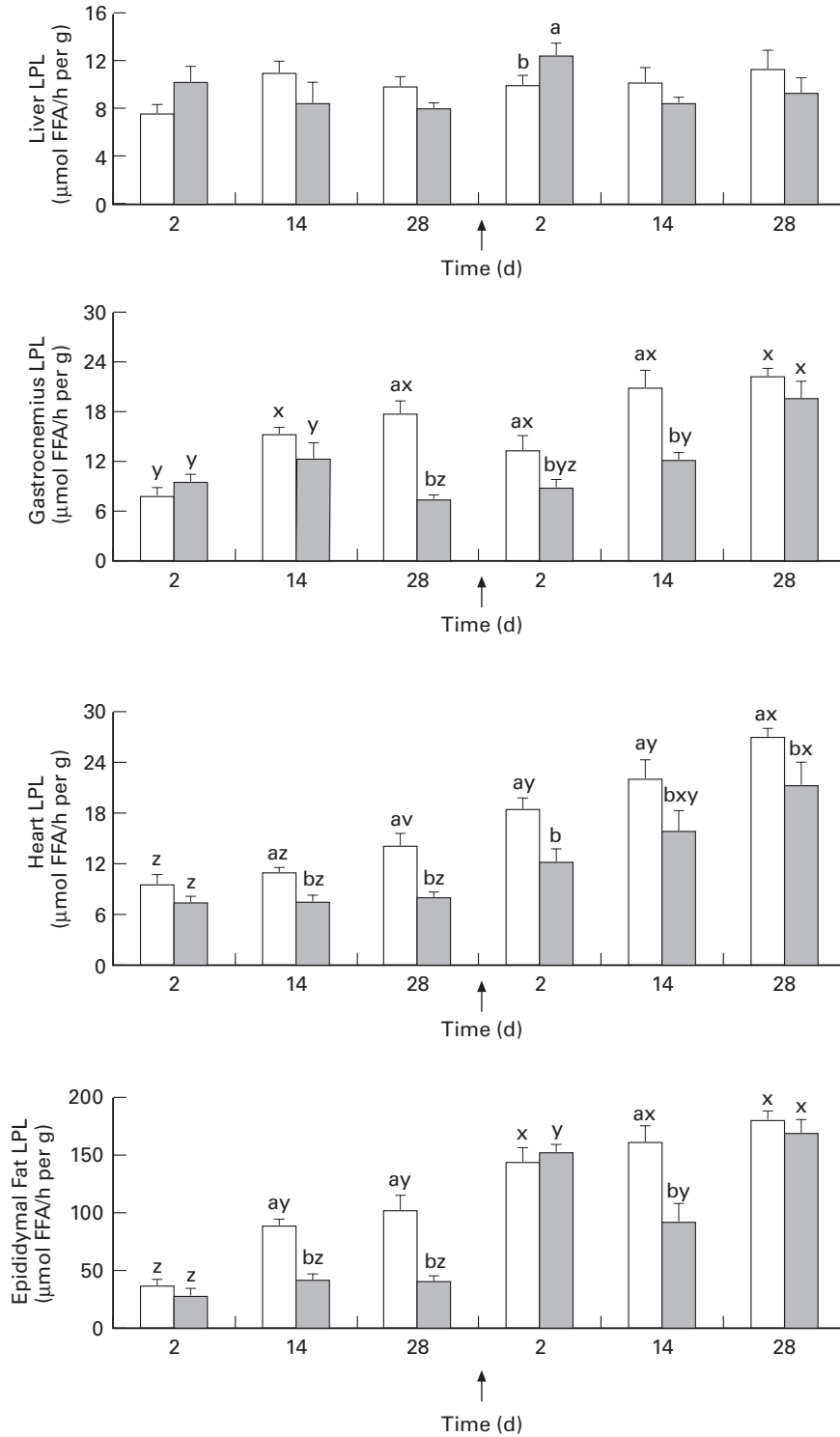
Fig. 3. Lipoprotein-lipase (LPL) activities of liver, gastrocnemius, heart and epididymal fat (expressed in μmol free fatty acids released/h per g fresh tissue). Values are means for six rats per group with their standard errors shown by vertical bars. The control group (□) was fed the balanced diet (160 g wheat gluten + 40 g casein/kg) for 56 d. The experimental group (▨) was fed the low-protein diet (40 g wheat gluten +10 g casein/kg) for 28 d (protein malnutrition) then was refed the balanced diet for 28 d (balanced refeeding). ↑, Change of diet. For details of composition of diets see Table 1. [a,b]Mean values were significantly different from those of the control group (two-way ANOVA and Duncan's multiple range test): $P < 0.05$. [x,y,z]Mean values were significantly different within a group over time (two-way ANOVA and Duncan's multiple range test): $P < 0.05$. [x,y,z]Mean values were significantly different within a group over time (two-way ANOVA and Duncan's multiple range test): $P < 0.05$.

Table 3. Liver, serum and VLDL-triacylglycerol (TAG) and VLDL-apolipoprotein (Apo) concentrations during protein malnutrition followed by balanced refeeding*

(Mean values with their standard errors for six rats per group)

	Protein malnutrition						Balanced refeeding					
	Day 2		Day 14		Day 28		Day 2		Day 14		Day 28	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Liver TAG (mmol/g)												
Control group	10·76[bx]	0·08	11·80[bx]	1·37	11·71[bx]	0·57	21·50[y]	0·57	17·28[az]	1·60	17·26[az]	2·55
Experimental group	19·95[ax]	2·31	46·38[ay]	10·03	56·98[ay]	14·05	18·94[xz]	6·82	11·39[bz]	1·72	8·46[bz]	1·10
Serum TAG (mmol/l)												
Control group	1·02[b]	0·09	0·78[a]	0·10	0·82[a]	0·06	0·75[b]	0·10	0·82[b]	0·09	0·82	0·07
Experimental group	1·22[ax]	0·03	0·51[by]	0·03	0·39[by]	0·10	1·31[ax]	0·18	0·91[ax]	0·10	0·91[x]	0·13
VLDL-TAG (mmol/l)												
Control group	0·74	0·10	0·73[a]	0·07	0·61[a]	0·24	0·54	0·25	0·79	0·03	0·77	0·16
Experimental group	0·64[x]	0·01	0·31[by]	0·01	0·19[bz]	0·03	0·64[x]	0·26	0·55[x]	0·17	0·57[x]	0·15
VLDL-Apo (g/l)												
Control group	0·16[x]	0·04	0·24[ax]	0·04	0·34[ay]	0·04	0·33[y]	0·01	0·39[y]	0·01	0·22[x]	0·05
Experimental group	0·17[x]	0·02	0·15[bx]	0·01	0·10[by]	0·01	0·32[z]	0·02	0·34[z]	0·03	0·27[z]	0·02

[a,b]Mean values within a column with unlike superscript letters were significantly different (two-way ANOVA and Duncan's multiple range test; $P < 0.05$).
[x,y,z]Mean values within a row with unlike superscript letters were significantly different (two-way ANOVA and Duncan's multiple range test; $P < 0.05$).
* The control group were fed the balanced diet (160 g wheat gluten +40 g casein/kg) for 56 d. The experimental group were fed the low-protein diet (40 g wheat gluten +10 g casein/kg) for 28 d (protein malnutrition) then refed the balanced diet for 28 d (balanced refeeding). For details of composition of diets see Table 1.

values with the control values at the same day of the experiment. During balanced refeeding, the body weights of experimental rats increased rapidly and markedly at the beginning, but 28 days of refeeding did not enable them to reach the body weight of the control rats, in spite of a similar food intake. Indeed, Mohamed-Benkada *et al.* (1993) have shown under similar experimental conditions that total recovery of body weight was obtained only after 3 months of balanced refeeding.

During protein malnutrition, the deficient group maintained constant LPL activity and relative weight of epididymal fat; however, when compared with the control group, these values were significantly decreased from day 14 to day 28 of protein malnutrition. Gastrocnemius and heart LPL activities were also stable during protein malnutrition but showed a reduction at day 28 of protein malnutrition when compared with control values. Only liver lipolytic activity remained unchanged during protein malnutrition. Similarly, Agbedana (1980) showed that adipose tissue LPL in malnourished rats was largely reduced, whereas changes in liver and heart lipolytic activities were not significant. It is suggested that these changes in LPL activities could be part of the mechanism of the development of fatty liver through the relative stability of HL activity which was necessary to TAG hydrolysis but the response to the reduced hepatic output of VLDL was the major part of this mechanism as shown previously (Meghelli-Bouchenak *et al.* (1989*b*). Indeed, in our experiment VLDL-Apo values were significantly reduced during protein malnutrition (Table 3) in the experimental group compared with control values. Rat serum lipoproteins did not show the same sensitivity to protein malnutrition. Indeed, in our previous paper (Meghelli-Bouchenak *et al.* 1989*b*) LDL and HDL were not affected by protein malnutrition. Moreover, Faergeman *et al.* (1975) established that most VLDL constituents in the rat are catabolised by the liver and only a small proportion is transferred into the LDL fraction. This has been put

forward as the explanation for the small plasma pool size of LDL in the rat compared with man in whom most VLDL are converted to LDL (Fidge & Poulis, 1978), whereas some studies carried out in children with kwashiorkor have shown a reduced level of these lipoproteins (Onitri & Boyo, 1975). Kadowaki *et al.* (1992) have shown that HL is necessary for the hepatic uptake of both HDL-TAG and cholesteryl esters. Moreover, in rats injected with antibody to endothelial HL, HDL-phospholipids and unesterified cholesterol are increased (Kuusi *et al.* 1979*a,b*). The unchanged HDL contents in association with the fall in VLDL concentrations during protein malnutrition (Meghelli-Bouchenak *et al.* 1989*a*) are surprising because additional Apo (especially ApoC and -E) and lipids are known to be transferred to HDL after hydrolysis of TAG in the core of TAG-rich lipoproteins (chylomicrons and VLDL) by LPL, producing larger HDL (Eisenberg, 1984). The hepatic TAG contents with the 40 g wheat gluten +10 g casein/kg diet were 4·86-fold higher than those with 160 g wheat gluten +40 g casein/kg, in spite of an increase in sucrose feeding (+24 g/kg). Liver TAG accumulation with the low-protein diet could have been chiefly due to overall amino-acid deficiency. The highly negative correlations observed between VLDL-Apo and liver TAG contents on the one hand, and the low concentration of VLDL-TAG (3·2-fold lower in the experimental group compared with control group) on the other hand, confirmed that hepatic steatosis was essentially due to impaired exportation and transport of TAG by VLDL. Indeed, there was no hypertriacylglycerolaemia despite the increase in sucrose in the diet. The unchanged hepatic lipolytic activity could participate in TAG accumulation in the liver during protein malnutrition. Moreover, the experimental group consuming more carbohydrates than controls (+24 g/kg) developed a fatty liver accompanied by a low concentration of VLDL-TAG.

Stable LPL activities of epididymal fat, gastrocnemius, heart and liver on the one hand, and the reduction in these

activities when compared with control group values on the other hand, were noted with this type of protein malnutrition. The lower activities were not sufficient to diminish the hydrolysis of the low level of VLDL-TAG, which would explain the low serum and VLDL-TAG concentrations. These data suggest that during protein malnutrition, the low available serum VLDL-TAG was probably sufficient to maintain the relative weight of epididymal fat at the level observed at the beginning of experiment and did not allow its increase, as observed in the control group. This could be explained by the fact that the low-protein diet limited lipid storage in adipose tissue from the low available serum VLDL-TAG, which could favour lipid utilisation by muscle and heart. Indeed, gastrocnemius and heart LPL activities were less reduced than those of epididymal fat in deficient rats and their relative weights remained unchanged during protein malnutrition and balanced refeeding.

In the rat, however, the effects of hypoinsulinaemia relative to protein deficiency (Lunn *et al.* 1983) on the low levels of LPL activity support the current interest in insulin resistance. It has been reported that fetal undernutrition may induce impaired β-cell development and insulin resistance (Barker *et al.* 1993; Barker, 1995). Impaired VLDL catabolism, reduced LPL activity, overproduction of TAG and ApoB-100 are common in adult diabetes mellitus (Ginsberg, 1991). Moreover, adipose tissue lipoprotein lipase activity is regulated by a mechanism involving insulin, whereas the heart enzyme is regulated differently (Borensztajn *et al.* 1972).

At the beginning of balanced refeeding, LPL activity of epididymal fat was increased and was similar to that of the control group. However, the relative and absolute weights of this tissue remained lower throughout refeeding duration. At day 14 of balanced refeeding, the epididymal fat tissue relative weight was increased compared with the value obtained at day 2 of balanced refeeding, but its LPL activity per g fresh tissue was diminished. LPL activities of heart and gastrocnemius were enhanced with refeeding duration but in contrast with LPL activity of epididymal fat tissue, these activities rose more progressively during balanced refeeding.

A rebound effect was observed only in liver lipolytic activity. This privileged synthesis of liver lipolytic activity during balanced refeeding indicates that lipolytic activity has its own particular modulated expression (transcription, glycosylation, transport and secretion). There is evidence for independent genetic regulation of LPL in different tissues (Ben-Zeev *et al.* 1983) and post-translational nutritional regulation of these LPL has been proposed (Doolittle *et al.* 1990).

In conclusion, our data confirm that protein malnutrition involves liver TAG accumulation, with reduced serum and VLDL-TAG and -Apo concentrations, i.e. impaired transport of TAG. LPL activities of the extrahepatic tissues studied were maintained at the values observed at the beginning of protein malnutrition, but when compared with those of control rats, these activities were lower and the lowest activity was found in epididymal fat. On the other hand, hepatic lipolytic activity did not change during protein malnutrition. Therefore, during protein malnutrition, the low VLDL-TAG exported from the liver could be hydrolysed efficiently by tissues despite the low extra-hepatic tissue LPL activity. However, though LPL activity is important in determining fatty acid availability to tissues, the substrate concentration is also critical. More direct studies of lipid utilisation are required since determination of lipolytic activities does not provide conclusive information on lipid fuel availability to tissues.

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