Depletion of Δ9 desaturase (*EC* 1.14.99.5) enzyme activity in growing rat during dietary protein restriction

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The effects of protein restriction on Δ9 desaturase (*EC* 1.14.99.5) activity were studied in growing rats. A control group was fed on a balanced diet (200 g casein/kg; BD) for 28 d. The experimental group was fed on the low-protein diet (20 g casein/kg; LP) for 26 d, then refed the balanced diet (BD-R) for 2 d. Rats were born to and suckled from normally fed dams. The enzyme activity was measured after 2 and 14 d of LP, and 26 d of LP plus 2 d of BD-R, by incubations in vitro of hepatic microsomal pellets with [1-^{14}C]stearic acid. The results indicated a decreased Δ9 desaturase activity after 2 and 14 d of LP of −33 and −43% respectively. Refeeding for 2 d was sufficient to super-repair this activity (+66%). The fatty acid composition of total liver lipids and microsomal phosphatidylethanolamines (PE) and phosphatidylcholines (PC) were also investigated; 18:0 decreased in total liver lipids at 14 d of LP, when 18:1*n*−9 increased. Stearic acid (18:0) increased in PC at 2 d of LP and in PE at 14 d of LP; oleic acid (18:1*n*−9) did not change. Therefore, it is concluded that a defect occurred in the bioconversion of 18:0 into 18:1*n*−9 by Δ9 desaturation during protein depletion. As oleic acid is accumulated in total liver lipids during LP, we speculate that this is due to a decreased oxidation or transport of this fatty acid.

Microsomal desaturation: Protein restriction: Liver lipids

Protein restriction alters the metabolism of polyunsaturated fatty acids (Waterlow *et al.* 1960; Williams & Hurlebaus, 1965*a*, *b*, 1966; Truswell *et al.* 1969; Flores *et al.* 1970; Naismith, 1973; Anthony & Edozien, 1975; Brenner, 1981; Holman *et al.* 1981; Leat, 1983). Rogers (1971, 1972) has shown that liver mitochondrial and microsomal polyunsaturated fatty acid compositions were altered by such a diet. All these findings suggested an impairment of the bioconversion of linoleic acid into arachidonic acid, via a decrease in desaturase activities.

Mercuri *et al.* (1979) showed that Δ6 and Δ5 desaturase activities were diminished in pregnant rats fed on a low-protein diet (50 g casein/kg, LP). Then De Tomas *et al.* (1980, 1983) reported decreased liver Δ6 and Δ5 desaturase activities in the early developing rat related to a maternal protein restriction. These authors also showed the negative effect of protein depletion on the supply of polyunsaturated fatty acids for normal development and metabolic adaptations. But Gerson & Wong (1978) indicated that, in the adult rat, if the liver fatty acid composition was affected by protein restriction, the Δ6 and Δ5 desaturase activities were not.

We have demonstrated, by a time-course study using a low-protein diet (50 g gluten/kg), the impairment of Δ6 and Δ5 desaturase activities in growing rats (Narce *et al.* 1986*a*). During protein depletion (20 g casein/kg) we noted a strong increase in oleic acid (18:1*n*−9) in total liver lipids (Narce *et al.* 1986*b*). Such an increase has been previously

shown in serum fatty acids (Taylor, 1971; Holman *et al.* 1981) and may be considered to reflect, at least partly, the situation in different tissues. Alterations have been shown in cholesterol and phospholipid (PL) content as well as in different PL classes of erythrocyte membranes obtained from children with kwashiorkor (Coward & Lunn, 1978; Fondu *et al.* 1980; Ramanadham & Kaplay, 1982). In similar conditions, Vajreswari *et al.* (1990) have shown that the proportion of palmitic acid (16:0) was significantly lower, and that of oleic acid significantly higher, in children with kwashiorkor than in controls. All these results suggest an impaired metabolism of palmitic, stearic and oleic acids, involving a possible defect in the hepatic desaturase enzyme system.

Previous findings reported a decreased Δ9 desaturase activity (enzyme converting stearic acid 18:0 into oleic acid 18:1*n*−9; *EC* 1.14.99.5) during pregnancy and early development, by feeding rats on a low-protein diet (Mercuri *et al.* 1979; De Tomas *et al.* 1980). On the other hand, Gerson & Wong (1978) demonstrated that Δ9 desaturase activity was not affected in adult rats fed on a protein-free diet.

Such inconsistent findings arose from different experimental conditions. None of these previous investigations was concerned with a post-weaning period in the growing rat, or took into account the fact that adaptive changes might occur during feeding of a low-protein diet (Narce *et al.* 1988*b*). On the other hand, Δ9 desaturase activity is known to have a different response from Δ6 and Δ5 desaturase activities to dietary manipulations (Brenner & Peluffo, 1974; Mercuri *et al.* 1979). Consequently, it appeared important to study, in growing rats, the liver microsomal Δ9 desaturation of 18:0 into 18:1*n*−9 under such nutritional conditions in order to clarify the inconsistencies in the literature and establish the role of dietary protein in the regulation of enzyme activity.

Thus, the present work investigates, in a time-course study in growing rats, the effects of a low-protein diet (20 g casein/kg) followed by a balanced refeeding diet on the activity of stearic acid Δ9 desaturase in the liver of rats born and suckled from normally fed dams.

Our results show a decreased Δ9 desaturase activity in growing rats after 2 and 14 d of LP diet, an unexpected result since the LP diet was high in carbohydrates known to stimulate Δ9 desaturation (Oshino & Sato, 1972). This decreased activity is less important than that of Δ6 and Δ5 desaturases (Narce *et al.* 1988*b*); Δ9 desaturase is known to be less responsive to dietary manipulations than other desaturases. The activity is super-repaired (i.e. increased above starting value) after 2 d of balanced refeeding. This low Δ9 desaturase activity is not related to the findings concerning the fatty acid composition of total liver; stearic acid decreased and oleic acid increased after 14 d of LP.

Therefore, the accumulation of oleic acid generally observed in liver, plasma and erythrocyte membrane during protein depletion may be due to a defective metabolism of this fatty acid only to the extent of decreased oxidation or transport, or both.

This work shows that the activity of the Δ9 desaturase is decreased during the post-weaning period as is that of the two other enzymes of fatty acid desaturation, Δ6 and Δ5 desaturases. This result is of importance for the understanding of fatty acid metabolism during feeding of a low-protein diet, especially during the growing period, as such a study has previously been lacking.

MATERIALS AND METHODS

Animals and diets

Twenty-four male Wistar rats (33 d of age; Iffa-Credo, l'Arbresle, France) weighing 110 (SE 5) g at the beginning of the experiment were fed on a balanced diet (200 g casein/kg) for 10 d and then divided into two groups. The control group (twelve rats) was fed on the balanced diet (BD) for 28 d while the experimental group (twelve rats) was fed on a low-

protein diet (LP; 20 g casein/kg) for 26 d and then the balanced diet (balanced refeeding; BD-R) for 2 d. The composition of the diets is shown in Table 1. Diets and tap water were supplied *ad lib.* and changed every other day. Rats were housed in stainless-steel cages at 25° with controlled 12 h light–dark cycle.

Isolation of microsomal pellets

Three rats of each group (LP and BD) were fasted for 12 h and killed, after the same duration of diet (2, 14 and 28 d), at 08.00 hours (in order to reduce the effect of possible diurnal variations in desaturase activities; Actis Dato *et al.* 1973) by cervical dislocation. These time intervals were chosen in order to study the first stage of LP and the immediate effect of refeeding after a period on a low-protein diet corresponding to our previous study (Narce *et al.* 1988*b*). The livers were quickly removed, rinsed with 0·15 M-sodium chloride, then weighed. Rat liver (3 g) was immediately cut into thin slices and homogenized at 4° in 18 ml of a cold solution containing 0·05 M-phosphate buffer pH 7·4 and 0·25 M-sucrose. The homogenate was centrifuged at 8000 ***g*** for 30 min, and the supernatant fraction then centrifuged at 105000 ***g*** for 60 min (Beckman L8-55 Ultracentrifuge; Beckman Instruments, Palo Alto, CA, USA). The protein content of the microsomal pellets resuspended in the buffer solution was determined by the method of Layne (1957).

In vitro enzyme assays

Incubations were carried out in open shaking flasks at 37° for 5 min; 5 mg microsomal protein were used in a total volume of 2·1 ml 0·15 M-phosphate buffer pH 7·4 containing (μmol): magnesium chloride 10, ATP 7·4, CoA 1, NADPH 25, [1-^{14}C]stearic acid 0·04. [1-^{14}C]stearic acid (60 mCi/mmol, 97% radiochemically pure) was purchased from the Radiochemical Centre, Amersham, UK, and diluted with the corresponding unlabelled fatty acid (Sigma, St Louis, MO, USA) to obtain an ethanol solution with a specific activity of about 5 mCi/mmol. The coenzymes and other reagents were pure products from Sigma or Merck (Darmstadt, Germany). Under such conditions the reaction was linear; % conversion = f(time and protein concentration) was measured from 3 to 15 min and from 20 to 60 nmol fatty acids (M. Narce and J-P. Poisson, unpublished results). The enzymic reaction was stopped by the addition of 5 ml potassium hydroxide (120 g/l). A control assay was done by adding microsomes and alcoholic potassium hydroxide at zero time of the reaction and no desaturase activity was observed. Then, the incubation mixtures were saponified for 1 h at 75° and fatty acids methylated according to Slover & Lanza (1979) after extraction with chloroform–methanol (10:1, v/v). The conversion of [1-^{14}C]stearic acid into [1-^{14}C]oleic acid (Δ9 desaturation) was measured using a Packard counter (Packard, Meriden, CT, USA) after separation of labelled fatty acids by high-performance liquid chromatography, according to Narce *et al.* (1988*a*), using a Waters chromatograph (Millipore, Molsheim, Germany) equipped with a Merck Lichrocart column (Merck, Darmstadt, Germany), and expressed as a percentage of control values.

Lipid and fatty acid analysis

Liver total lipids were extracted according to the Delsal (1944) method using methylal–methanol (4:1, v/v), then evaluated gravimetrically. The rate of liver neutral lipid formation was obtained from the difference between total lipids and phospholipids (PL). The amount of total PL was determined by the method of Bartlett (1959).

The fatty acid composition of liver total lipids was measured by gas–liquid chromatography (model 419 gas–liquid chromatograph; Packard; equipped with a 30 m capillary glass column packed with Carbowax 20M) after lipid extraction, saponification and methylation as previously described (Narce *et al.* 1988*b*). Liver microsomal L-α-

Table 1. *Composition of diets by weight (g/kg) and energy %*

	Protein (Milk casein*)	Fat (Peanut oil†)	Carbohydrates	
			Maize starch‡	Sucrose*
Balanced diet				
Wt (g/kg)	200	60	600	50
Energy (%)	20·3	13·7	60·9	5·0
Low-protein diet				
Wt (g/kg)	20	60	780	50
Energy (%)	2·03	13·7	79·2	5·0
	Minerals§	Vitamins ‖	Fibre agar-agar*	
Balanced diet				
Wt (g/kg)	20	20	50	
Low-protein diet				
Wt (g/kg)	20	20	50	

The two diets were semi-synthetic, isoenergetic (16845 kJ/kg diet) and were given as a powder.

* Prolabo, Paris, France.

† Peanut oil was a commercial product. Fatty acid composition of peanut oil (g/100 g total fatty acids): palmitic acid (16:0) 11·6, stearic acid (18:0) 3·1, oleic acid (18:1$n-9$) 46·5, linoleic acid (18:2$n-6$) 31·4, arachidic acid (20:0) 1·5, gondoic acid (20:1$n-9$) 1·4, eicosa-8,11-dienoic acid (20:2$n-9$) 0·1, behenic acid (22:0) 3·0.

‡ Louis François, St Maur, France.

§ UAR 205 B, Epinay sur Orge, France. The salt mix provided the following (mg/kg diet): calcium 3000, phosphorus 2100, potassium 1830, chloride 1200, sodium 900, magnesium 214, manganese 40, zinc 12, copper 4·5, cobalt 0·32, iodine 0·9, iron 105.

‖ The UAR 200 vitamin mixture provided the following (mg/kg diet): retinol 51·6, cholecalciferol 1, thiamin 150, riboflavin 200, pyridoxin 70, cyanocobalamine 0·2, calcium pantothenate 250, nicotinic acid 800, pteroylmonoglutamic acid 20, vitamin E 500, menadione 30, choline 175.

phosphatidylcholines (PC) and L-α-phosphatidylethanolamines (PE) were isolated by thin-layer chromatography according to the method of Wagner *et al.* (1961), and their fatty acid composition was analysed as described previously. Results were reported as arithmetical means for each group with their standard errors. The significance of differences between assays and controls was determined by the unpaired Student's *t* test.

RESULTS

Rat body- and liver weights, liver weight:body-weight values, total liver lipids, neutral lipids and phospholipid amounts are presented in Table 2. The BD group showed a normal body-weight increase, while the LP group weighed 52% of the BD group, after 14 d of LP. After 26 d of LP then 2 d of BD-R the rats still weighed only 49% of the BD group. The relative liver weight of the LP group, obtained by liver weight:body-weight ratio, was lower than that of the BD group after 2 d on the diet (−17%), but not significantly different after 14 d of LP or 26 d of LP followed by 2 d of BD-R. The liver total lipid content was unchanged after 2 d of LP diet, but significantly increased after 14 d. After 2 d of BD-R the liver total lipid content was still increased compared with the BD group. The neutral-lipid weights changed in the same way, but the phospholipid content remained unchanged during the duration of the experiment.

The effect of diet on stearic acid Δ9 desaturation is shown in Fig. 1. The enzyme activities are expressed as a percentage of control values, each control value being 100. Control values were 633 (SE 19·5) pmol/mg protein per min. The animals fed on the LP diet showed

Table 2. *The effects of dietary protein restriction on body- and liver weights and liver triacylglycerol and phospholipid contents of growing rats*

(Mean values with their standard errors for three animals)

Dietary regimen†...	2 d BD		2 d LP		14 d BD		14 d LP		28 d BD		2 d BD-R	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Body-wt (g)	135·7	7·6	125·0	4·2	205·0	10·8	106·3**	2·1	244·0	2·8	120·3**	3·4
Liver-wt (g)	6·97	0·7	5·36*	0·4	9·60	1·2	4·70*	0·3	10·9	0·6	5·90**	0·3
$\frac{\text{Liver-wt}}{\text{body-wt}} \times 100$	5·13	0·3	4·28*	0·2	4·70	0·4	4·40	0·2	4·50	0·3	4·90	0·2
Total lipids (mg/g liver)	49·5	4·1	44·7	3·0	53·9	3·7	72·0*	6·0	47·7	2·5	67·1*	5·4
Neutral lipids (mg/g liver)	24·8	3·2	21·7	4·4	29·3	4·8	45·4*	6·7	22·4	3·9	40·9**	4·3
Phospholipids (mg/g liver)	24·7	5·3	23·0	2·8	24·6	8·0	26·6	6·3	25·7	4·7	26·2	1·3

BD, balanced diet; LP, low-protein diet; BD-R, balanced refeeding.
Mean values were significantly different from control values (BD): * $P < 0·05$, ** $P < 0·01$.
† For details, see Table 1 and pp. 628–629.

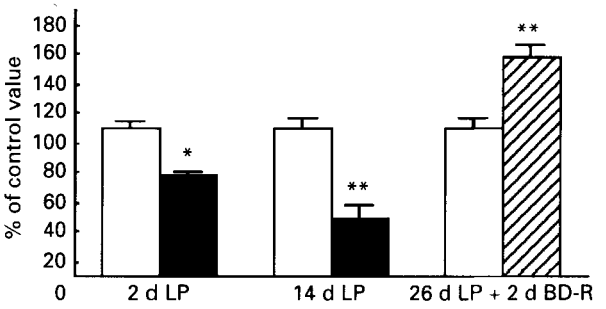

Fig. 1. The effects of dietary protein restriction on Δ9 desaturation of [1-^{14}C]stearic acid in hepatic microsomes of rats. (□), balanced diet (BD; 2, 14 and 28 d); (■), low-protein diet (LP; 2 and 14 d); (▨), balanced refeeding (BD-R; 2 d). For details of dietary regimen, see Table 1 and pp. 628–629. The values are means, expressed as a percentage of control (BD) values, with their standard errors represented by vertical bars for three animals. Mean values were significantly different from those for controls (BD): * $P < 0·05$, ** $P < 0·01$.

a decreased Δ9 desaturase activity after 2 and 14 d on this diet (−33 and −43% respectively). The diminished Δ9 desaturase activity was more marked after 14 d than after 2 d of LP. A super-repaired Δ9 desaturase activity was observed after 2 d of BD-R (+66%).

The fatty acid composition of microsomal PC and PE in the microsomal fraction is shown in Tables 3 and 4. Stearic acid was increased significantly (+17%) in PC at 2 d and in PE at 14 d (+36%) of LP. Palmitoleic acid (16:1$n-7$) decreased, but not significantly, in PC and PE. Oleic acid remained unchanged in PC and PE during the administration of LP. These two fatty acids (palmitoleic and oleic) are products of Δ9 desaturation. The $n-6$ fatty acid compositions were more markedly changed in PE than in PC; 18:2$n-6$ increased after 2 d on the LP diet (+17%), but significantly decreased after 14 d (−41%). Arachidonic acid (20:4$n-6$) decreased significantly (−27%) at the beginning of the experiment, and remained lower (but not significantly) than that of the control after 14 d of LP feeding. In PC, 18:2$n-6$ was increased after 2 d of the experiment (+26·5%), but no significant change appeared after 14 d. The 20:4$n-6$ content did not change in rats fed

Table 3. *The effects of dietary protein restriction on fatty acid composition of hepatic microsomal* L-α-*phosphatidylcholine (mg/100 mg total fatty acids) of rats*

(Mean values with their standard errors for three animals)

Dietary regimen†...	2 d BD		2 d LP		14 d BD		14 d LP		28 d BD		2 d BD-R	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
16:0	29·6	1·0	30·6	2·3	25·4	1·1	24·8	2·7	18·7	1·5	22·9	2·0
16:1*n*−7	2·3	0·2	1·6	0·2	3·0	0·2	2·6	0·7	2·1	0·2	2·4	0·2
18:0	24·5	0·7	28·6*	0·8	16·5	1·9	17·7	1·4	21·2	0·7	20·7	1·0
18:1*n*−9	14·9	0·3	13·5	1·1	15·3	1·3	15·9	1·0	10·8	1·2	12·2	2·3
18:2*n*−6	10·6	0·1	13·4*	0·8	13·3	1·8	10·5	0·2	12·3	0·7	6·7**	1·1
18:3*n*−6	0·2	0·01	0·17	0·02	traces		traces		0·3	0·03	0·6*	0·1
20:3*n*−6	1·1	0·1	1·7*	0·1	traces		traces		2·6	0·4	1·5*	0·2
20:4*n*−6	13·7	1·4	13·1	4·7	21·8	1·1	22·7	1·9	29·6	0·6	27·3	2·8
22:5*n*−6	1·2	0·5	1·0	0·9	0·2	0·3	1·4	0·3	1·1	0·7	4·1*	1·0
22:6*n*−3	1·9	0·3	1·0	0·7	2·6	0·4	4·6**	0·3	1·3	0·8	1·8*	0·3
20:4*n*−6:18:2*n*−6	1·3	0·1	0·98	0·2	1·7	0·09	2·16	0·1	2·4	0·1	4·1	0

BD, balanced diet; LP, low-protein diet; BD-R, balanced refeeding.
Mean values were significantly different from control values (BD): * $P < 0·05$, ** $P < 0·01$.
† For details, see Table 1 and pp. 628–629.

Table 4 *The effects of dietary potein restriction on fatty acid composition of hepatic microsomal* L-α-*phosphatidylethanolamines (mg/100 mg total fatty acids) of rats*

(Mean values with their standard errors for three animals)

Dietary regimen†...	2 d BD		2 d LP		14 d BD		14 d LP		28 d BD		2 d BD-R	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
16:0	25·3	1·8	24·5	2·0	24·9	4·9	23·9	2·8	21·0	5·0	24·3	3·6
16:1*n*−7	1·3	0·1	1·1	0·01	4·0	1·0	2·6	0·6	1·4	0·3	2·2	0·6
18:0	29·2	2·5	34·7	2·2	16·8	0·1	22·9**	0·7	23·3	2·6	22·4	1·1
18:1*n*−9	10·4	0·8	8·9	0·4	13·7	0·1	11·3	1·7	5·4	0·9	6·2	0·6
18:2*n*−6	8·1	0·5	9·5*	0·2	9·1	0·1	5·4**	0·4	7·9	0·4	4·0**	0·03
18:3*n*−6	0·2	0·02	0·3	0·02	traces		traces		0·2	0·01	0·3*	0·01
20:3*n*−6	1·2	0·1	1·4	0·3	traces		traces		1·8	0·2	1·1	0·2
20:4*n*−6	22·2	0·4	16·3**	1·4	25·3	2·9	19·0	0·2	33·4	4·7	28·7	1·7
22:5*n*−6	1·1	0·1	0·4**	0·1	1·0	1·0	2·2	1·6	2·2	1·0	8·3*	1·5
22:6*n*−3	0·8	0·1	3·8**	0·3	5·3	1·8	6·0	3·7	3·5	1·0	3·1	1·0
20:4*n*−6:18:2*n*−6	2·7	0·3	1·7*	0·2	2·8	0·3	3·5	0·4	4·2	0·4	7·1	0·3

BD, balanced diet; LP, low-protein diet; BD-R, balanced refeeding.
Mean values were significantly different from control values (BD): * $P < 0·05$, ** $P < 0·01$.
† For details, see Table 1 and pp. 628–629.

on the LP diet at the different times of our study. The 22:6*n*−3 content increased significantly in PE after 2 d of LP (+375%) and in PC after 14 d of experiment (+77%).

After 2 d of BD-R no change was observed for 18:0 and 18:1*n*−9 levels in PC and PE. The 18:2*n*−6 levels remained lower than those of the controls in PE (−49%) and in PC (−45%). The 20:4*n*−6 content was not different from that of the controls and 22:6*n*−3 remained higher after the refeeding period, only in PC (+38%).

On the other hand, the fatty acid composition of total liver lipids (Fig. 2) showed a significant increase in the amount of 16:0 after 14 d of LP feeding. 18:0 decreased after 2

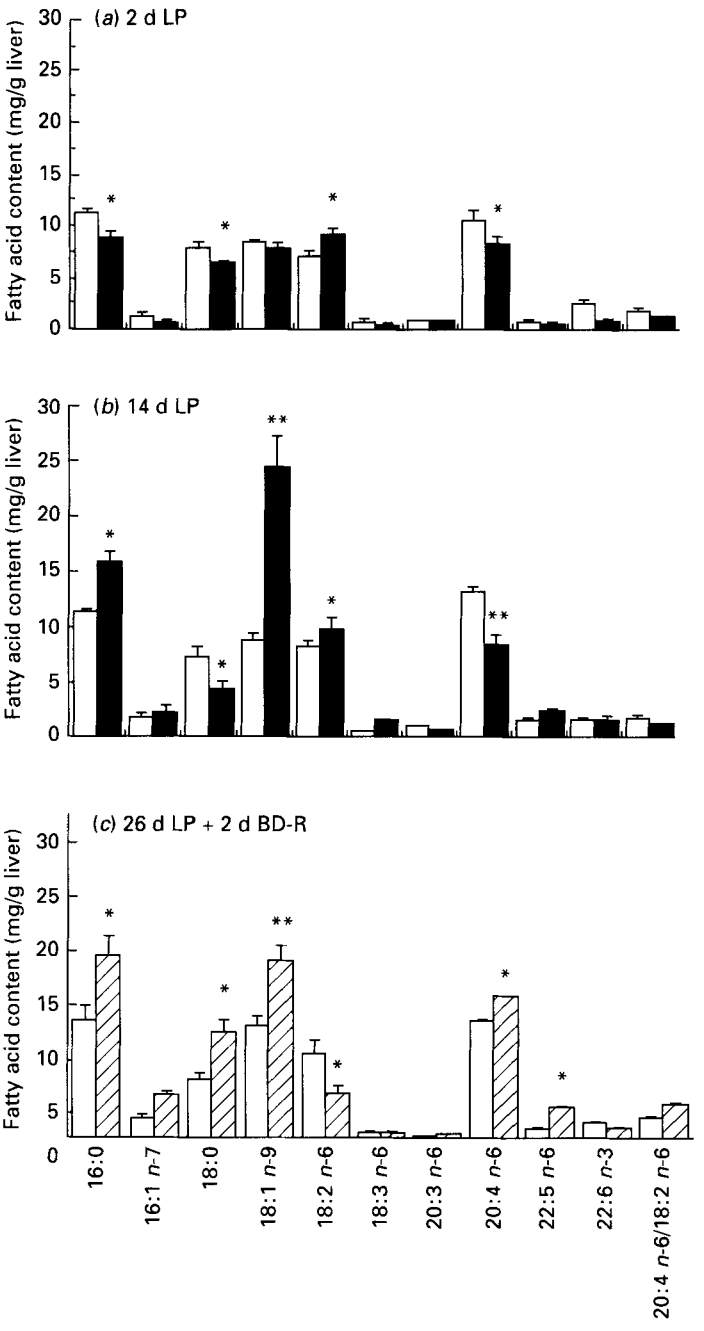

Fig. 2. The effects of dietary protein restriction of fatty acid composition of liver total lipids (mg/g liver) of rats. (□), balanced diet (BD; 2, 14 and 28 d); (■), low-protein diet (LP; 2 and 14 d); (▨), balanced refeeding (BD-R; 2 d). For details of dietary regimen, see Table 1 and pp. 628–629. The values are means with their standard errors represented by vertical bars for three animals. Mean values were significantly different from those for controls (BD): * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$.

and 14 d of LP, and 18:1$n-9$ increased after 14 d, being 2·5 times higher in the LP group when compared with the BD group, as previously described by Narce *et al.* (1988*b*). 18:2$n-6$ increased significantly after 2 d of LP, and remained significantly higher than control after 14 d of experiment. 20:4$n-6$ decreased after 2 d of experiment, and was significantly lower than control (−34%) after 14 d. The ratio of 20:4$n-6$ to 18:2$n-6$ decreased after 2 and 14 d of the experiment. Feeding BD-R for 2 d was not sufficient to reverse the effect of LP on the 18:0 and 18:1$n-9$ status in total liver lipids in spite of a super-repaired effect on the level of 18:0 (twofold higher than that of controls); 18:1$n-9$ diminished but remained twofold higher than the BD value. A reversed effect was observed on the $n-6$ polyunsaturated fatty acids; 18:2$n-6$ decreased and 20:4$n-6$ increased after 2 d of BD-R, but these values were not significantly different from those of the controls.

DISCUSSION

With regard to the animal weights, the results obtained in the present experiment confirm our previously reported findings (Narce *et al.* 1986*a*) observed in spite of a non-significant difference in food intake between the LP and BD groups (69 *v.* 79 g/kg body-weight). The relative liver weight of the LP group (liver weight:body-weight ratio) was lower than that of the BD group after 2 d of the experiment (−17%), but not significantly different after 14 d. The increased liver total lipid content observed in the LP group after 14 d of experiment, essentially due to the neutral lipids, is well known (Meghelli-Bouchenak *et al.* 1987, 1989*a*; Fernandez Ortega, 1989); we previously reported no change in the PL content (Narce *et al.* 1988*b*). Meghelli-Bouchenak *et al.* (1989*b*) have shown that the PL concentration was decreased after 28 d of protein restriction (20 g casein/kg or 50 g gluten/kg).

Fig. 1 shows clearly that Δ9 desaturation is consistently depleted by the LP diet during the growth period (−33 and −43%). Narce *et al.* (1988*b*) have reported that during dietary protein restriction animals adapt themselves to the LP regimen and, consequently, Δ6 and Δ5 desaturase appeared to be more or less decreased at different intervals of feeding the diet (these activities were decreased by about 70% after 2 d, 10% after 14 d, and 50% after 52 d of the feeding regimen); Fig. 1 shows that Δ9 desaturase is less sensitive to such a phenomenon than the other desaturases. The super-repaired Δ9 desaturase activity that was observed after 26 d LP then 2 d BD-R (+66%) emphasizes that the impairment of Δ9 desaturase activity is reversible by balanced refeeding, as are Δ6 and Δ5 desaturase activities (Narce *et al.* 1986*b*).

The immediate effect of changing the diet (both LP and BD-R) was more pronounced for Δ6 than for Δ9 desaturase activity; the Δ9 desaturase activity was −33 and +66% after 2 d of LP and BD-R respectively, compared with −80 and +88% for Δ6 desaturase activity. Δ9 desaturase is known to be less responsive to dietary manipulations than other desaturases (Brenner & Peluffo, 1974; Mercuri *et al.* 1979). Decreased Δ9 desaturase activity was shown by Mercuri *et al.* (1979) in pregnant rats and by De Tomas *et al.* (1980) in pups from dams fed on deficient diets throughout gestation until 31 d of age. Our results show clearly that Δ9 desaturase activity is still depleted by protein restriction after this age, this activity, nevertheless, not being affected in adult rats (Gerson & Wong, 1978); a sudden protein restriction stops the development of rats and impairs Δ9 desaturase activity during growth, as during pregnancy, when the protein needs are high for the biosynthesis of animal tissues. This result was obtained in spite of a higher level of carbohydrate in the LP diet, which is known to have an activating effect on Δ9 desaturase (Inkpen *et al.* 1969; Oshino & Sato, 1972). This change has to be considered as a sign of a reduction of Δ9 desaturase synthesis or modifications of enzymic properties, or both.

The fatty acid composition of PE seemed to be more sensitive to the duration of the LP diet than that of PC. These findings are not in agreement with those of Rogers (1972) who showed a decreased 18:0 and an increased 18:1*n*−9 content in microsomal PL after 6·5 months on a 40 g casein/kg diet. This may be due to differences in experimental conditions between the two groups of investigators. However, a high proportion of 18:0 was found in liver PL of pups from dams fed on a LP diet throughout gestation and until 31 d of age (De Tomas *et al.* 1980). In 49-d-old rats, Medwadowski & Lyman (1973) showed (with an 80 g casein/kg diet fed for 7 weeks) an increased 18:0 and a decreased 18:1*n*−9 content in microsomal PE and PC. These findings suggest a negative effect of protein depletion during growth on the conversion of 18:0 to 18:1*n*−9 in rat liver microsomal fractions.

The fatty acid composition of total liver lipids (Fig. 2) is not in agreement with the findings for microsomal PL composition in PC and PE (Tables 3 and 4) and the decreased Δ9 desaturase activity (Fig. 1). Williams & Hurlebaus (1966) and Gerson & Wong (1978) found the same increase of 18:1*n*−9 in adult rats, in spite of a lack of effect on Δ9 desaturase activity. Similar results were obtained in plasma (Holman *et al.* 1981) and in erythrocyte membrane (Brown *et al.* 1978; Vajreswari *et al.* 1990), reflecting at least partly the situation in the liver.

Our findings indicate that the high level of oleic acid observed in different tissues is not due, even partly, to a defect of the hepatic Δ9 desaturation. This change may be due to a depletion of the other enzyme systems involved in lipid metabolism such as fatty acid synthetase (*EC* 6.2.1.3), acyltransferase (*EC* 2.3.1.40), hydroxybutyrate dehydrogenase (*EC* 1.1.1.30), or the enzyme systems of incorporation and oxidation (Gerson & Wong, 1978). The removal of fatty acids from the liver is also altered (Truswell *et al.* 1969; Seakins & Waterlow, 1972; Yagasaki & Kametaka, 1978; Meghelli-Bouchenak *et al.* 1987). Consequently, we may speculate that 18:0 levels decreased at 14 d of LP because of a depletion of its endogenous synthesis system, and that 18:1*n*−9 levels increased because of a depletion of its catabolism or removal systems, or both. The accumulation of oleic acid may be reinforced by its high proportion in the diet (Table 1), but Gerson & Wong (1978) have observed a similar result with maize oil, less rich in oleic acid.

The refeeding of a balanced diet shows that this impairment of lipid metabolism due to protein depletion is progressively reversible.

CONCLUSION

Until now, findings concerning the effects of protein restriction on Δ9 desaturation were inconsistent (Gerson & Wong, 1978; Mercuri *et al.* 1979; De Tomas *et al.* 1980, 1983) and information about the effect of such a diet on Δ9 desaturation during growth was lacking.

In the present work a time-course study with young rats fed on a low-protein diet was an original approach for protein restriction, providing evidence that Δ9 desaturase activity is impaired, as indeed are Δ6 and Δ5 desaturase activities, during growth under such a dietary regimen. Such a finding is related to the slight changes observed in fatty acid composition of microsomal PE and PC. Thus, the decreased 18:0 and increased 18:1*n*−9 levels in total liver lipids that we observed at 14 d of LP are not related to Δ9 desaturase activity as previously postulated (Narce *et al.* 1988*b*). Consequently, we may conclude that these changes are due only to modifications of enzyme systems involved in the synthesis of 18:0 or the catabolism or transport of 18:1*n*−9, or both. This investigation of the time-course of Δ9 desaturase activity in growing rats improves our knowledge of endogenous fatty acid metabolism during protein depletion. Balanced refeeding reverses rapidly all the noted effects on desaturation, but 2 d of refeeding are not sufficient to restore the PC- and PE-fatty acid composition to control values.

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