Characterization of the stimulatory effect of high-fat diets on peroxisomal β -oxidation in rat liver

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1. The effect on rat liver peroxisomal β -oxidation of feeding diets containing various amounts of dietary oils was investigated. With increasing amounts (5–25%, w/w) of soya-bean oil an apparent, but not statistically significant, increase of 1.5-fold was found both in specific activity, and in total liver activity. Increasing amounts of partially hydrogenated marine oil revealed a sigmoidal dose–response-curve, giving a 4–6-fold increase in the peroxisomal β -oxidation activity at 20% or more of this oil in the diet. 2. Addition of small amounts of soya-bean oil to the marine-oil diet had no effect on the peroxisomal β -oxidation activity, but decreased the $C_{20:3(5,8,11)}$ fatty acid/ $C_{20:4(5,8,11,14)}$ fatty acid ratio in liver phospholipids from 0.74 to 0.01. 3. Starvation for 2 days led to a 1.5–1.8-fold increase in the peroxisomal β -oxidation activity in rats previously fed on a standard pelleted diet, but had no effect in rats given high-fat diets. 4. Feeding partially hydrogenated marine oil or partially hydrogenated rape-seed oil resulted in higher activities than the corresponding unhydrogenated oils. 5. No significant differences in the effect on peroxisomal β -oxidation could be detected between diets containing rape-seed oils with 15 or 45% erucic acid respectively. 6. These findings are discussed in relation to the possible effects of $C_{22:1}$ and *trans* fatty acids in the process leading to increased peroxisomal β -oxidation activity in the liver.

Several conditions have been described which influence the activity of liver peroxisomal β -oxidation. It has been found to be increased in starvation (Ishii *et al.*, 1980a), in diabetes (Horie *et al.*, 1981; Snoswell & Fishlock, 1980), after treatment with various hypolipidaemic drugs, such as clofibrate (Lazarow & de Duve, 1976) or triadenol (Berge & Bakke, 1981), and also after intake of the widely used plasticizer di-(2-ethylhexyl) phthalate (Osumi & Hashimoto, 1978).

In rat liver, high-fat diets also cause an increase in peroxisomal β -oxidation (Neat *et al.*, 1980; Ishii *et al.*, 1980b), and diets rich in very-long-chain mono-unsaturated fatty acids seem to produce the most marked induction (Neat *et al.*, 1980). A role has thus been proposed for peroxisomal β -oxidation as regards oxidation of fatty acids during periods of sustained high hepatic influx of these compounds, and especially in relation to the metabolism of fatty acids that are poorly oxidized by mitochondrial β -oxidation (Neat *et al.*, 1981).

Evidence is also accumulating that an increase in peroxisomal β -oxidation activity in the liver may be one of the main events explaining the transient

nature of the lipid accumulation in the heart of rats given diets rich in 22:1 fatty acids (Christophersen *et al.*, 1982; Bremer & Norum, 1982).

In the present study we have tried to further characterize the increase in peroxisomal β -oxidation activity due to high-fat diets, and to correlate the extent of increase to the fatty acid composition of the dietary oils.

Experimental

Materials

Dietary oils, including their analytical data, were obtained from DeNoFa og Lilleborg Fabriker A/S, Fredrikstad, Norway, except for the unhydrogenated rape-seed oils, which were supplied by AB Karlshamns Oljefabriker, Karlshamn, Sweden. The fatty acid compositions are shown in Table 1. Clofibrate [ethyl 2-(4-chlorophenoxy)-2-methylpropionate] was obtained from Weiders Farmasøytiske A/S, Oslo, Norway. Other reagents were commercial products of high purity, mostly from Sigma Chemical Co., St. Louis, MO, U.S.A.

Table 1. *Fatty acid composition of dietary oils*

Only major fatty acids (1% or more) are included in the table. The marine oils were obtained from capelin (*Mallotus villosus*). The rape-seed oil with about 15% C_{22:1} fatty acid ('RO₁₅') was obtained by mixing a high-erucic acid and a low-erucic acid rape-seed oil.

Fatty acid composition in each dietary oil (%)

Fatty acid	Marine oil	Partially hydrogenated marine oil	'RO ₁₅ ' rape-seed oil	Rape-seed oil	Partially hydrogenated rape-seed oil	Soya-bean oil	Partially hydrogenated soya-bean oil
C _{14:0}	6.7	6.8					
C _{16:0}	14.7	12.8	4.2	4.9	4.1	10.9	10.6
C _{16:1}	9.5	9.9					
C _{18:0}	1.0	3.0	1.6	1.0	6.3	3.8	14.0
C _{18:1}	16.1	15.1	44.7	13.5	32.5	24.3	73.8
C _{18:2}	1.6	2.5	19.6	20.0		52.5	
C _{18:3}			8.1	7.9		7.5	
C _{20:0}		2.4	1.0		1.7		
C _{20:1}	12.8	16.4	3.8	6.2	6.5		
C _{20:2}	5.4	4.3					
C _{20:5}	9.0						
C _{22:0}		3.0			5.6		
C _{22:1}	13.5	14.5	15.8	43.5	42.7		
C _{22:6}	6.2						
Proportion of <i>trans</i> double bonds (%)		55			65		55

Animals and diets

Weanling male rats of the Wistar strain (body weight approx. 60g) were purchased from Møllegaard breeding laboratory, Ejby, Denmark. Semisynthetic diets were prepared as described by Thomassen *et al.* (1979), increasing amounts of dietary oils being corrected for by decreasing amounts of corn starch. The rats were fed on a standard pelleted diet for 5 days, and then given the experimental diets for 3 weeks. They were kept two and two in grid-bottomed cages, and had, if not otherwise stated, free access to food and water. For more details about housing conditions, see Thomassen *et al.* (1979). Some animals were treated with clofibrate for 10–12 days. The clofibrate was dissolved in acetone and mixed with the diet (0.3%, w/w), and the acetone was then evaporated by air.

Preparation of the 10% (w/v) liver homogenate and the peroxisome-enriched fraction

The rat was stunned and decapitated, and the liver was minced with scissors and homogenized in a medium consisting of 0.25 M-sucrose, 1 mM-EDTA and 2 mM-potassium phosphate buffer, pH 7.4, using a Teflon/glass homogenizer at 720 rev./min. The resulting homogenate was centrifuged at 3200 g_{av.}-min to remove debris, the pellet homogenized once

more, centrifuged and the combined supernatants were diluted to give the 10% (w/v) homogenate.

A peroxisome-enriched fraction was prepared from this homogenate by centrifugation at 150 000 g_{av.}-min. The resulting pellet was resuspended in the homogenization medium to give a protein concentration of about 10 mg/ml. This fraction contained 90–95% of the palmitoyl-CoA-dependent NAD⁺-reduction activity found in the 'parent' 10% (w/v) homogenate.

Enzyme assays

Palmitoyl-CoA-dependent NAD⁺ reduction was measured spectrophotometrically as described by Lazarow (1977), with the modifications introduced by Flatmark *et al.* (1981). A Cary double-beam spectrophotometer model 219 was used, and the contents of the sample and reference cuvette were identical except for palmitoyl-CoA, which was added to the sample cuvette only.

Lipid extraction and g.l.c. analysis

Lipid extraction and separation of lipid classes were performed essentially as described previously (Thomassen *et al.*, 1979). Fatty acids in the phospholipid fraction were methylated by using a mixture of benzene, methanolic HCl and dimethoxypropane at room temperature overnight (Mason & Waller, 1964).

The methyl esters were separated on a non-polar (SE 30) vitreous silica capillary column in a Carlo Erba Fractovap 2150 Gas Chromatograph equipped with a flame-ionization detector. Carrier gas flow was 0.75 ml/min, split ratio 1:100. Injector and detector temperature was 275°C. Splitless injection was performed at 50°C, and column temperature was programmed (LT programmer model 232) to rise rapidly to 220°C (40°C/min) and then by a slow gradient (0.5°C/min) to 260°C. Peak areas were measured by a Hewlett-Packard 3390 reporting integrator. Identification of major peaks was made by comparing the retention times with those of fatty acid methyl ester standards obtained from Supelco Inc., Bellefonte, PA, U.S.A.

Protein determination

Protein was determined by using the Folin-Ciocalteu reagent (Lowry *et al.*, 1951) with bovine serum albumin as standard.

Statistical analysis

Student's *t* test was used to evaluate the significance of differences between population means. Probabilities greater than 0.05 were taken to be non-significant.

Results

Effects of increasing amounts of dietary oils

In previous studies (Neat *et al.*, 1980, 1981) high-fat diets (15% by wt.) containing C_{22:1} fatty acids were found to result in a higher peroxisomal β -oxidation activity than diets containing the more 'ordinary' oils, such as soya-bean oil and peanut oil. For this reason we chose to compare the effects of increasing amounts of a partially hydrogenated

marine oil containing about 15% C_{22:1} fatty acids with that of soya-bean-oil-based diets. The rats were fed for 3 weeks on diets containing various amounts of the two oils as described in the Experimental section. The weight gain and food consumption of the rats fed on various amounts of partially hydrogenated marine oil diets are given in Table 2. No significant differences could be detected among the different dietary groups, except for the group receiving 30% (w/w) of the oil, in which a slightly (about 20%) lower food consumption and weight gain was observed.

The effect of various amounts of dietary oils on the cyanide-insensitive β -oxidation activity in the peroxisome-enriched fraction is presented in Table 3. As found also in the previous study with isolated peroxisomes (Neat *et al.*, 1980) a slight, but not

Table 2. *Effect of various amounts of partially hydrogenated marine oil on food consumption and growth of the rat*

Rats were fed on the diets indicated for 3 weeks, with free access to food and water. The tabulated values represent means $\pm$ s.d. for the numbers of animals indicated in parentheses. Abbreviation used: PHMO, partially hydrogenated marine oil.

PHMO proportion of diet (% w/w)	Food consumption (g/day)	Weight gain (g/day)
5 (4)	19.7 $\pm$ 2.5	5.1 $\pm$ 0.3
10 (4)	16.5 $\pm$ 2.2	4.8 $\pm$ 0.4
15 (4)	18.7 $\pm$ 1.5	5.6 $\pm$ 0.3
20 (4)	17.7 $\pm$ 1.5	5.0 $\pm$ 0.7
25 (4)	19.0 $\pm$ 2.6	5.4 $\pm$ 1.3
30 (4)	13.8 $\pm$ 0.8	4.1 $\pm$ 0.5

Table 3. *Effect of various amounts of dietary oils on liver peroxisomal β -oxidation activity*

Rats were fed on the diets indicated for 3 weeks. Peroxisome-enriched fractions were prepared from the livers, and assayed for cyanide-insensitive palmitoyl-CoA-dependent NAD⁺ reduction as described in the Experimental section. The tabulated values represent means $\pm$ s.d. for the numbers of animals indicated in parentheses. Abbreviations used: PHMO, partially hydrogenated marine oil; SO, soya-bean oil.

Dietary composition (% w/w)	NADH [nmol $\cdot$ min ⁻¹ $\cdot$ (mg of protein) ⁻¹]	NADH [nmol $\cdot$ min ⁻¹ $\cdot$ (whole liver) ⁻¹]	NADH [nmol $\cdot$ min ⁻¹ $\cdot$ (g of liver) ⁻¹]
PHMO			
5 (4)	5.34 $\pm$ 0.75	1026 $\pm$ 123	90.3 $\pm$ 10.1
10 (4)	5.47 $\pm$ 0.18	1101 $\pm$ 100	125.3 $\pm$ 58.2
15 (4)	6.47 $\pm$ 2.37	1274 $\pm$ 443	116.5 $\pm$ 37.9
20 (4)	12.78 $\pm$ 2.84	2402 $\pm$ 803	212.5 $\pm$ 81.8
25 (4)	13.18 $\pm$ 2.75	2846 $\pm$ 1176	233.0 $\pm$ 111.0
30 (4)	14.15 $\pm$ 2.48	3431 $\pm$ 940	308.6 $\pm$ 100.7
SO			
5 (4)	2.45 $\pm$ 1.17	598 $\pm$ 369	59.2 $\pm$ 53.0
15 (4)	2.93 $\pm$ 0.99	635 $\pm$ 221	57.3 $\pm$ 16.2
25 (3)	3.84 $\pm$ 0.58	1046 $\pm$ 261	93.3 $\pm$ 14.7

statistically significant, enhancement was observed after increasing the amount of soya-bean oil from 5 to 15% (w/w). The effect was not markedly increased when 25% of this oil was included in the diet. Thus, only a small increase in the peroxisomal β -oxidation activity was detected in this study due to a high-fat diet when soya-bean oil was the source of the dietary fat. After feeding partially hydrogenated marine oil, however, higher values were found even with 5% oil in the diet, and the increase in activity was significantly greater than in the soya-bean-oil-fed animals, especially between 15 and 20%. Above 20% there seemed to be little further increase. Thus, with the marine oil in the high-fat diet a maximal stimulation of about four times is observed when compared with a low-fat diet also containing the marine oil, or about six times when compared with soya-bean-oil-based low-fat diet. Similar effects were apparent when the results were expressed per g of liver or per whole liver, and also when total liver homogenates were used as the enzyme source (results not shown).

Effects of starvation and clofibrate administration

In this experiment an attempt was made to correlate the effects of fat feeding to that of other treatments reported to increase peroxisomal β -oxidation. Rats were fed for 3 weeks on diets containing either 25% (w/w) of soya-bean oil or partially hydrogenated marine oil, or (as a control) a standard pelleted diet, and then subjected to starvation for 2 days (48 h). The effects on the peroxisomal β -oxidation activity are shown in Table 4. With animals given a low-fat (standard pelleted) diet, starvation led to a 1.5–1.8-fold increase in the peroxisomal β -oxidation activity when related to mg of protein or calculated as total liver capacity respectively. This is in good agreement with previous

observations made by Ishii *et al.* (1980a). With high-fat diets, however, no significant changes could be detected, neither with soya-bean oil nor with the marine oil, except when the results were expressed per g of liver. In this case, starvation led to higher values in all groups, most probably due to decreased amounts of liver glycogen. These results indicate that starvation and fat feeding may exert their effects on peroxisomal β -oxidation through a common factor.

A study on the effect of administering the highly potent inducer of peroxisomal β -oxidation activity, clofibrate, was also included, to compare its effects with that of high doses of the marine oil. Table 4 shows that the specific activity was only slightly higher in the peroxisome-enriched fraction from the clofibrate-treated animals than from the animals given 25% (w/w) marine oil. The total liver capacity, on the other hand, was about 2.5 times higher after the clofibrate treatment than after feeding marine oil.

Effects of essential fatty acids

Essential-fatty-acid deficiency is known to give rise to several changes in the lipid metabolism of rat liver (Holman, 1968). The widely different effects on peroxisomal β -oxidation of increasing amounts of soya-bean oil and partially hydrogenated marine oil could thus be related to the marginal supply of essential fatty acids through the marine oil diet. In animals fed 20% (w/w) marine oil for 3 weeks a strong decrease in the percentage of $C_{18:2(9,12)}$ and $C_{20:4(5,8,11,14)}$ fatty acids in total liver phospholipids was observed when compared with animals fed 25% (w/w) soya-bean oil (Table 5). An accumulation of $C_{20:3(5,8,11)}$ fatty acid was also apparent, giving a $C_{20:3(5,8,11)}$ fatty acid/ $C_{20:4(5,8,11,14)}$ fatty acid ratio of 0.74 in the marine-oil-fed group.

Table 4. *Effects of starvation and clofibrate treatment on liver peroxisomal β -oxidation*

Rats were fed on semisynthetic diets containing 25% (w/w) of soya-bean oil (SO) or partially hydrogenated marine oil (PHMO), or on a standard pelleted diet with or without clofibrate, as described in the Experimental section. The animals designated 'starved' were deprived of food for 48 h, but had free access to drinking water. Peroxisome-enriched fractions from the livers were prepared and assayed for cyanide-insensitive palmitoyl-CoA-dependent NAD⁺-reduction as described in the Experimental section. Values represent means $\pm$ s.d. for the numbers of animals indicated in parentheses. Statistically significant differences between starved and non-starved animals are indicated by: *, $0.05 > P > 0.01$; †, $0.01 > P$.

Diets	NADH [nmol $\cdot$ min ⁻¹ $\cdot$ (mg of protein) ⁻¹]	NADH [nmol $\cdot$ min ⁻¹ $\cdot$ (whole liver) ⁻¹]	NADH [nmol $\cdot$ min ⁻¹ $\cdot$ (g of liver) ⁻¹]
PHMO (4)	10.53 $\pm$ 0.15	1883 $\pm$ 221	212.7 $\pm$ 18.9
PHMO, starved (4)	10.10 $\pm$ 1.68	2290 $\pm$ 144	284.3 $\pm$ 24.8†
SO (4)	4.86 $\pm$ 1.33	1275 $\pm$ 145	119.7 $\pm$ 17.9
SO, starved (4)	4.85 $\pm$ 1.65	1108 $\pm$ 553	152.0 $\pm$ 25.0*
Pellets (4)	2.71 $\pm$ 0.47	646 $\pm$ 140	55.0 $\pm$ 13.0
Pellets, starved (4)	4.03 $\pm$ 0.34†	1139 $\pm$ 211†	129.5 $\pm$ 23.6†
Pellets + clofibrate (4)	14.35 $\pm$ 0.50	5125 $\pm$ 101	581.4 $\pm$ 123.1

Table 5. Fatty acid composition of liver phospholipids (%)

Rats were fed on semisynthetic diets containing 20% (w/w) partially hydrogenated marine oil (PHMO), 20% (w/w) partially hydrogenated marine oil + 5% (w/w) soya-bean oil (SO), or 25% (w/w) soya-bean oil for 3 weeks. Liver phospholipids were isolated and analysed by g.l.c. as described in the Experimental section. Values represent means $\pm$ s.d. for the number of animals indicated in parentheses. Statistically significant differences from the PHMO + 5% (w/w) SO group are indicated by: *, $0.05 > P > 0.01$; †, $0.01 > P$.

Fatty acid	Fatty-acid composition of phospholipids in each dietary group (%)		
	PHMO (4)	PHMO + 5% (w/w) SO (4)	25% (w/w) SO (4)
C _{14:0}	0.2 $\pm$ 0.1	0.3 $\pm$ 0.1	0.2 $\pm$ 0.1
C _{16:0}	15.7 $\pm$ 1.4†	13.2 $\pm$ 0.9	13.9 $\pm$ 1.8
C _{16:1}	3.1 $\pm$ 0.1†	2.5 $\pm$ 0.3	0.3 $\pm$ 0.1†
C _{18:0}	19.4 $\pm$ 1.0†	15.8 $\pm$ 1.4	27.5 $\pm$ 2.1†
C _{18:1}	24.6 $\pm$ 2.1†	19.2 $\pm$ 0.9	7.5 $\pm$ 0.6†
C _{18:2(9,12)}	7.6 $\pm$ 0.7†	16.6 $\pm$ 1.1	14.5 $\pm$ 2.6
C _{18:3}	2.3 $\pm$ 0.2†	0.4 $\pm$ 0.1	0.1 $\pm$ 0.1
C _{20:0}	0.4 $\pm$ 0.1†	0.2 $\pm$ 0.1	Trace
C _{20:1}	1.9 $\pm$ 0.3†	1.5 $\pm$ 0.1	0.2 $\pm$ 0.1
C _{20:2}	1.7 $\pm$ 0.5*	0.4 $\pm$ 0.1	0.9 $\pm$ 0.2†
C _{20:3(8,11,14)}	2.0 $\pm$ 0.3	2.5 $\pm$ 0.3	0.7 $\pm$ 0.2†
C _{20:3(5,8,11)}	3.6 $\pm$ 0.4†	0.1 $\pm$ 0.1	Trace
C _{20:4(5,8,11,14)}	4.9 $\pm$ 0.5†	14.0 $\pm$ 1.1	20.3 $\pm$ 4.1*
C _{22:0}	1.2 $\pm$ 0.1	1.0 $\pm$ 0.2	0.3 $\pm$ 0.1†
C _{22:1}	0.3 $\pm$ 0.1	0.2 $\pm$ 0.1	Trace
C _{22:5}	Trace	0.5 $\pm$ 0.1	0.2 $\pm$ 0.1*
C _{22:6}	3.0 $\pm$ 0.7†	5.0 $\pm$ 0.2	3.3 $\pm$ 1.4
C _{20:3(5,8,11)/C_{20:4(5,8,11,14)}}	0.74 $\pm$ 0.09†	0.01 $\pm$ 0.002	< 0.01
C _{20:3(8,11,14)/C_{18:2(9,12)}}	0.26 $\pm$ 0.02†	0.15 $\pm$ 0.02	0.04 $\pm$ 0.01†
C _{20:4(5,8,11,14)/C_{20:3(8,11,14)}}	2.48 $\pm$ 0.32†	5.65 $\pm$ 1.08	29.00 $\pm$ 9.60†

The use of the ratio of C_{20:3(5,8,11)/C_{20:4(5,8,11,14)}} fatty acids as a measure of essential-fatty-acid deficiency has been disputed when long-chain and *trans* fatty acids are included in the diet (Lundberg, 1980). The value of 0.74 found in the animals given partially hydrogenated marine oil for 3 weeks is, however, well above 0.40, which is considered an upper limit of the range of normality (Holman *et al.*, 1964), and may still be taken as an indication that a slight essential-fatty-acid deficiency had developed. No skin symptoms or marked weight reduction were, however, apparent. When the marine-oil diet was supplied with linoleic acid by adding 5% soya-bean oil, the percentage of C_{18:2(9,12)} and C_{20:4(5,8,11,14)} fatty acids increased about two and three times respectively; C_{18:2(9,12)} fatty acid reached the level found in soya-bean-oil-fed animals, and C_{20:4(5,8,11,14)} fatty acid was still slightly decreased. The C_{20:3(5,8,11)} fatty acid was hardly detectable, decreasing the C_{20:3(5,8,11)/C_{20:4(5,8,11,14)}} fatty acid ratio to 0.01, only slightly higher than in soya-bean-oil-fed animals. This indicates that 2.5% (w/w) linoleic acid is sufficient to prevent, at least for shorter feeding periods, the development of essential-fatty-acid deficiency also in cases where diets

containing appreciable amounts of very-long-chain and *trans* (55%) fatty acids are fed.

The same extent of increase in peroxisomal β -oxidation was, however, found in the two partially-hydrogenated-marine-oil-fed groups (Table 6). Thus, the low supply of essential fatty acids does not seem to be responsible for the remarkably high peroxisomal β -oxidation activity observed in the rats receiving marine-oil diets. The partially-hydrogenated-marine-oil feeding also affected the product/precursor ratios in the linoleate sequence. Thus an increase in the C_{20:3/C_{18:2}} and a decrease in the C_{20:4/C_{20:3}} fatty acid ratios, compared with the soya-bean-oil-fed animals, was apparent. This effect was only partly abolished by the addition of soya-bean oil. Similar effects have been observed also by Hølmer (1980), but the reason for this relative accumulation of C_{20:3(8,11,14)} fatty acid after partially-hydrogenated-marine-oil feeding is at present not readily understood.

Effects of hydrogenation

An experiment was designed in an attempt to elucidate the possible role of *trans* fatty acids in the dietary oils. The rats were fed for 3 weeks, as before,

Table 6. *Effects of essential fatty acids on liver peroxisomal β -oxidation*

Rats were fed on semisynthetic diets containing 20% (w/w) of partially hydrogenated marine oil (PHMO), or 20% (w/w) PHMO + 5% (w/w) soya-bean oil (SO) for 3 weeks. Peroxisome-enriched fractions were prepared from the livers and assayed for cyanide-insensitive palmitoyl-CoA-dependent NAD⁺ reduction as described in the Experimental section. Values are means $\pm$ s.d. for the numbers of animals indicated in parentheses. The differences between the values in the two groups were statistically non-significant ($P > 0.05$).

Dietary oil	NADH [nmol $\cdot$ min ⁻¹ $\cdot$ (mg of protein) ⁻¹]	NADH [nmol $\cdot$ min ⁻¹ $\cdot$ (whole liver) ⁻¹]	NADH [nmol $\cdot$ min ⁻¹ $\cdot$ (g of liver) ⁻¹]
PHMO (4)	12.0 $\pm$ 2.1	2041 $\pm$ 640	178 $\pm$ 71
PHMO + SO (4)	10.0 $\pm$ 0.5	2282 $\pm$ 483	188 $\pm$ 30

Table 7. *Effect of different dietary oils on liver peroxisomal β -oxidation*

Rats were fed on semisynthetic diets containing 25% (w/w) of the different oils (for fatty acid composition, see Table 1) for 3 weeks. Peroxisome-enriched fractions were prepared and assayed for cyanide-insensitive palmitoyl-CoA-dependent NAD⁺ reduction as described in the Experimental section. Values are means $\pm$ s.d. for the numbers of animals indicated in parentheses. Statistically significant differences between the values for the animals given the partially hydrogenated or the unhydrogenated oil are indicated by: *, $0.05 > P > 0.01$; †, $0.01 > P$. Abbreviations used: RO₁₅, rape-seed oil with about 15% C_{22:1} fatty acid; RO, rape-seed oil; PHRO, partially hydrogenated rape-seed oil; MO, marine oil; PHMO, partially hydrogenated marine oil; SO, soya-bean oil; PHSO, partially hydrogenated soya-bean oil.

Dietary oil	NADH [nmol $\cdot$ min ⁻¹ $\cdot$ (mg of protein) ⁻¹]	NADH [nmol $\cdot$ min ⁻¹ $\cdot$ (whole liver) ⁻¹]	NADH [nmol $\cdot$ min ⁻¹ $\cdot$ (g of liver) ⁻¹]
RO ₁₅ (4)	5.48 $\pm$ 1.82	1221 $\pm$ 556	101.0 $\pm$ 49.9
RO (4)	6.89 $\pm$ 1.31	1656 $\pm$ 350	148.4 $\pm$ 54.9
PHRO (4)	11.05 $\pm$ 2.77*	2742 $\pm$ 372†	249.7 $\pm$ 30.4†
MO (4)	7.00 $\pm$ 0.17	1921 $\pm$ 107	156.2 $\pm$ 12.6
PHMO (4)	13.18 $\pm$ 2.75†	2846 $\pm$ 476†	233.0 $\pm$ 51.0†
SO (3)	3.84 $\pm$ 0.58	1046 $\pm$ 261	93.3 $\pm$ 14.7
PHSO (3)	6.13 $\pm$ 1.15†	1227 $\pm$ 296	99.4 $\pm$ 23.5

on diets containing 25% (w/w) rape-seed oil, marine oil or soya-bean oil, or with the corresponding partially hydrogenated oils. The percentage of *trans* double bonds was about the same (from 55 to 65; see Table 1). Feeding unhydrogenated marine oil or rape-seed oil resulted in lower activities than feeding the corresponding partially hydrogenated oils, whether the results were expressed per mg of protein, per g of liver or per whole liver (Table 7). The unhydrogenated soya-bean oil also seemed to give a somewhat lower value than the partially hydrogenated oil, when calculated on a mg-of-protein basis in the peroxisome-enriched fraction. This difference disappeared, however, when the results were related to liver weight or whole liver. A slightly different protein composition in the peroxisome-enriched fraction may thus be responsible for the apparent difference in the peroxisomal β -oxidation activity in these two groups.

Effect of C_{22:1} fatty acids

The most marked increase in peroxisomal β -oxidation has been observed with diets containing C_{22:1} fatty acids (Neat *et al.*, 1980, 1981). As shown

in Table 7, however, the extent of increase was equally high with the marine oils containing about 15% C_{22:1} fatty acid as with the rape-seed oils containing about 45% C_{22:1} fatty acid.

We therefore also included an experiment with rats fed on a diet containing a rape-seed oil with about 15% C_{22:1} fatty acid. As shown in Table 7, this diet resulted in a peroxisomal β -oxidation activity similar to that observed with the high-erucic acid rape-seed oil and with the unhydrogenated marine oil. Even though C_{22:1} fatty acids in the diet seem to be of importance, no simple and direct relationship between the content of C_{22:1} fatty acid and the extent of increase can readily be observed.

Discussion

The present results show that the effects of high-fat diets on peroxisomal β -oxidation activity in the rat liver comprise a complex picture as regards doses and composition of the dietary oils.

Most high-fat diets tested (i.e. rape-seed oil, partially hydrogenated marine oil, soya-bean oil and ground-nut oil) have been reported to result in

enhanced peroxisomal β -oxidation (Ishii *et al.*, 1980b; Neat *et al.*, 1980, 1981), although Lawson *et al.* (1981) found no increase with corn oil or beef tallow. The extent of the increase is, however, very much dependent on the type of dietary oil. Partially-hydrogenated-marine-oil diets lead to strong increases in this activity, and in this study a sigmoidal dose-response pattern was observed (Table 3). To what extent the β -oxidation activity found in the rats fed on diets containing 20% or more of partially hydrogenated marine oil, represents the maximal obtainable activity due to fat feeding is at present unknown. No other dietary oils tested in our laboratory, however, have induced higher values of peroxisomal β -oxidation activity. Administration of the hypolipidaemic drug clofibrate resulted in a similar high specific activity (Table 4), but seems to differ from the partially-hydrogenated-marine-oil feeding in other ways. Thus a much higher total liver capacity is obtained (Table 4), probably due to peroxisomal proliferation (Flatmark *et al.*, 1981), which is found only to a minor extent with the partially-hydrogenated-marine-oil diets (Christiansen *et al.*, 1981), and to increased liver weight (Kurup *et al.*, 1979).

In agreement with previous studies (Neat *et al.*, 1980, 1981), the more marked changes in the peroxisomal β -oxidation activity due to high-fat feeding was found with oils containing C_{22:1} fatty acids. In spite of this, no direct relationship seems to exist between the amounts of these acids in the diet and the extent of the increase. This is in contrast with the effects observed in heart, where an increase in the peroxisomal β -oxidation activity directly proportional to the dietary content of erucic acid was found (J. Norseth & M. S. Thomassen, unpublished work). Thus, in the liver other factors may act as 'inducing agents', or by modifying the response to C_{22:1} fatty acids. An essential-fatty-acid deficiency enforced by a high amount of *trans* fatty acids in the diet has been suggested to be of importance in this respect (Christiansen *et al.*, 1979). However, the results presented in this study clearly dissociate the effect of partially hydrogenated marine oil on the peroxisomal β -oxidation activity from that of a low supply of essential fatty acids. *Trans* fatty acids, on the other hand, seem to influence the response to dietary oils in the liver. From the experiment presented in Table 7 it might be concluded that *trans* isomers of very-long-chain fatty acids are more potent in increasing peroxisomal β -oxidation than the corresponding *cis* isomers. This also correlates well with our proposal of a role for peroxisomes in the oxidation of *trans* fatty acids in the liver (Neat *et al.*, 1981). This conclusion is, however, somewhat obscured by the effects of rape-seed oil on rat liver found by Christiansen *et al.* (1980). A time-course study of peroxisomal β -

oxidation revealed a transitory stimulation after 4 days on a rape-seed-oil diet followed by a gradual decrease with prolonged feeding. Thus, with rape-seed oil the effect in liver may be the result of two opposing mechanisms; a stimulatory effect on peroxisomal β -oxidation and an additional, at present unknown, inhibitory effect. Abolishment of the inhibitory effect of rape-seed oil by hydrogenation might also offer an explanation for the results presented in Table 7. No further information regarding the effects of hydrogenated rape-seed oil on liver peroxisomal β -oxidation is, however, at present available. To what extent the unhydrogenated marine oil has a similar effect is also still an open question.

Very little is known about the cellular events leading to the increase in peroxisomal β -oxidation, although high levels of acyl-CoA, which is slowly oxidized by the mitochondria, has been suggested to be of importance (Christophersen *et al.*, 1982; Bremer & Norum, 1982). In support of this idea metabolic conditions such as starvation (Table 4), high-fat feeding (Tables 3 and 7) and diabetes (Horie *et al.*, 1981) giving rise to increased peroxisomal β -oxidation, were also shown to result in an increased level of acyl-CoA in the liver (Tubbs & Garland, 1964).

The physiological role of the peroxisomal β -oxidation system is poorly understood. It is, however, believed to be of importance in the adaptation to diets rich in C_{22:1} fatty acids (Christophersen *et al.*, 1982). Thus an increase in peroxisomal β -oxidation activity results in a more extensive chain-shortening of C_{22:1} fatty acids (Christiansen, 1978; Neat *et al.*, 1981), giving rise to higher amounts of the more easily metabolized C_{18:1} fatty acids. Consequently, our finding that the partially hydrogenated oils resulted in higher activities than the corresponding unhydrogenated oils may offer an explanation as to why brassidic acid (the *trans*-isomer of erucic acid) is found to give rise to less lipidosis than erucic acid in the heart of weanling rats (Astorg, 1981). The adaptation process, however, is most probably also a consequence of changes in fatty acid metabolism taking place in several organs; thus the effect of C_{22:1} fatty acid-containing diets on peroxisomal β -oxidation activity in heart and small intestine requires further investigation.

In conclusion the highest increase in peroxisomal β -oxidation of the liver is found with dietary oils rich in C_{22:1} fatty acids. Hydrogenation of the oils seemed to be especially important for the increase in this activity, whereas essential-fatty-acid deficiency was not found to be correlated with the increased β -oxidation.

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