review

Metabolism of very long-chain monounsaturated fatty acids (22:1) and the adaptation to their presence in the diet

J. Bremer and K. R. Norum[1]

Institute of Medical Biochemistry and Institute for Nutrition Research, University of Oslo, Oslo, Norway

Abstract Unadapted rats and other animal species have a limited capacity to metabolize monounsaturated fatty acids with 22 carbons (22:1). Excess amounts in the diet of fats containing these fatty acids cause a transient accumulation (lipidosis) of triacylglycerol in the heart and other tissues but not in the liver, which seems to export the 22:1 fatty acids as very low density lipoproteins to the blood plasma. The acute lipidosis most probably is explained by a slow oxidation of 22:1 acyl-CoA by the mitochondrial acyl-CoA dehydrogenase combined with an inhibitory effect of this CoA ester on the oxidation of acyl-CoA esters of a more "normal" chain length. Other fatty acid metabolizing enzymes also show slow reaction rates with the 22:1 fatty acids. Upon continued feeding of diets with 22:1 fatty acids, an adaptation takes place and the lipidosis disappears. This adaptation coincides with the development of an increased capacity to chain-shorten the 22:1 fatty acids, especially in the liver, but also in the heart. The chain-shortening seems to be due to a partial β-oxidation of the 22:1 fatty acids by the peroxisomal β-oxidation enzyme system which shows an increased activity in adapted rats. In such rats, less 22:1 fatty acids circulate in the plasma very low density lipoproteins than in unadapted rats. The drug clofibrate (ethyl-*p*-chlorophenoxyisobutyrate) which induces increased activity of the peroxisomal β-oxidation enzymes, provides partial protection against the lipidosis in unadapted animals. Hydrogenated fish oil (containing different 22:1 isomers and many fatty acids with *trans* double bonds) is more efficient as an inducer of the chain-shortening of erucic acid in the liver than is rapeseed oil, which contains only one 22:1 fatty acid isomer and no fatty acids with *trans* double bonds. The hydrogenated fish oil causes less lipidosis than does rapeseed oil when diets containing the same amount of 22:1 fatty acids are fed. It is suggested that CoA esters that are poorly oxidized by the mitochondria (e.g., esters of erucic acid, of some fatty acids with *trans* double bonds, and of clofibric acid) may trigger the adaptation process.—**Bremer, J., and K. R. Norum.** Metabolism of very long-chain monounsaturated fatty acids (22:1) and the adaptation to their presence in the diet. *J. Lipid Res.* 1982: **23:** 243–256.

Supplementary key words rapeseed oil • fish oil • cetoleic acid • erucic acid • *trans* fatty acids • β-oxidation • peroxisomes • mitochondria • carnitine • lipoproteins • triacylglycerols • heart lipidosis • clofibrate

In 1970 Abdellatif and Vles (1) reported that rats fed a diet rich in rapeseed oil developed a substantial cardiac lipidosis. This accumulation of triacylglycerols (TG) was temporary. However, a rapeseed oil diet also causes focal necrosis and fibrosis in the heart (2). These findings and subsequent studies have been treated in several reviews (3–6).

The large, but transient accumulation of fat in the heart of rats ingesting the 22:1 diet and the adaptation to it raised several interesting biochemical and physiological questions which have been studied by several groups over the last 10 years. These studies have shown that the metabolism of 22:1 fatty acids is different in several ways from the metabolism of "normal length" fatty acids, and the studies also have revealed previously unknown adaptation mechanisms in the metabolism of fatty acids. These studies are the subject matter of the present review.

DIETARY SOURCES OF THE 22:1 FATTY ACIDS

The main sources of 22:1 fatty acids in the human diet are vegetable oils from rapeseed and mustard seed. The 22:1 isomer in these seeds is erucic acid (*cis* Δ13-docosenoic acid). Fish oils (from herring, capelin, and several other species) also contain 22:1 fatty acids. The most abundant of these acids is cetoleic acid (*cis* Δ11-docosenoic acid). When the fish oils are partially hydrogenated, which is necessary for their use as edible fat,

Abbreviations: TG, triacylglycerols; 22:1, monounsaturated fatty acids with 22 carbon atoms; VLDL, very low density lipoproteins.

[1] To whom reprint requests should be addressed at: Institute for Nutrition Research, University of Oslo, P.O. Box 1046, Blindern, Oslo 3, Norway.

several 22:1 isomers are formed and a large fraction of these isomers have a *trans* double bond (7).

The annual world production of edible fats and oils is about 53×10^9 kg. Approximately, 7.0% of this is rapeseed oil and 2.3% is oil of marine origin. It is difficult to assess the amount of 22:1 ingested by humans at present, since the possibility of health hazards from the 22:1 fatty acids has led to the development of new cultivars of rapeseed with low or no content of erucic acid. Furthermore, variations in sources of fish and in hydrogenation techniques can alter the content of 22:1 isomers of marine origin (8).

In Norway, relatively large amounts of hydrogenated marine oil are used as edible fat, and the average annual consumption of 22:1 has been calculated to be about 0.9 kg per person, i.e., approx. 0.8% of the nutrient energy.

DIGESTION AND ABSORPTION

The fat in diets containing large amounts of 22:1 fatty acids in the form of rapeseed oil or partially hydrogenated herring oil are more slowly digested and absorbed than other fats (9, 10). All studies on apparent digestibility of 22:1 fatty acids have been carried out with rapeseed oil with a high content of erucic acid. The reported capacity to digest and absorb 22:1 varies between approximately 60 and 99% depending on animal species and the amount of rapeseed oil in the diet. The lower values have been found in the Sprague-Dawley rat, rabbit, sheep, and guinea pig (11–14), while the higher values have been reported for the Wistar rat, chicken, dog, pig, and man (11, 14–18).

Studies on the composition of intestinal lymph of rats fed rapeseed oil showed that the percentage of erucic acid in lymph TG was about 70% of that of the dietary oil (19). This may be due to a lower digestibility of the erucic acid as compared to the other fatty acids in this oil. It is also a possibility that a metabolic conversion of the 22:1 fatty acids to shorter monoenes takes place in the intestinal wall. Microperoxisomes, which may shorten very long chain fatty acids (see below), have been reported to be present in the intestine of rat and guinea pig (20). Studies on human chyle have, however, revealed the same ratio of erucic acid in dietary and chyle fat (21).

THE MYOCARDIAL LIPIDOSIS CAUSED BY 22:1 FEEDING

The accumulation of heart lipids has been demonstrated in most species tested, including monkeys, but there are species differences. Thus, pigs seem to be more resistant to heart lipidosis than rats (22, 23). The lipidosis is related to the amount of 22:1 in the diet and is produced by the 22:1 of both vegetable and marine origin, although the accumulation of lipids in rats is most likely less for the marine isomers than for erucic acid (4, 24).

The accumulated heart lipids are almost exclusively TG. No increase in phospholipids or cholesteryl esters has been found (6, 25, 26). One can observe intracellular accumulation of TG as early as 3 hr after the ingestion of 22:1-containing oils (27).

The fatty acid composition of the accumulated lipids in the heart partially reflects the composition of the diet. It should be noted, however, that heart lipids contain less 22:1 and more 18:1, 18:0, and 16:0 than the diet (**Table 1**) after a feeding period of 3 days, 1 week, and 3 weeks (24, 28). The TG accumulation is most pronounced in the muscle cells of the heart. The lipidosis is also present in other organs, but not in the liver (4).

Cardiac muscle cells preferentially oxidize fatty acids. It is therefore tempting to attribute the acute TG accumulation to an inhibition of fatty acid oxidation, although other explanations have been suggested.

The accumulation of TG in the myocardium is obviously an imbalance between the net input and the oxidation of fatty acids (see **Fig. 1**). Free fatty acids (A in Fig. 1) in the blood are transported to the tissues as albumin complexes. It is known that the affinity of albumin for fatty acids decreases when the fatty-acid/albumin ratio is increased. This change in affinity plays an important role in the delivery of fatty acids to individual tissues. Very long chain fatty acids are bound more loosely to albumin than ordinary fatty acids. Thus, albumin has an affinity for erucic acid about 1/3 of that for palmitic acid (29). This might cause an increased erucic acid uptake in the tissues when a diet containing large amounts of 22:1 fatty acids is fed. This, however, should result in cardiac TG with more 22:1 than is absorbed from the diet, which is probably not the case (Table 1).

An increased activity of lipoprotein lipase (B in Fig. 1) may also increase input of fatty acids to heart muscle. Hülsman et al. (30) suggested that the significant increase of lipoprotein lipase activity observed in rat heart after 4 days on a rapeseed oil diet may contribute to the lipid accumulation. This would also contribute to the increased concentration of free fatty acids in hearts of rats fed rapeseed oils rich in 22:1 (31).

However, the activity of heart lipoprotein lipase increases upon olive oil feeding (32) which does not lead to TG accumulation in the heart. Lipoprotein lipase, therefore, can be of significance only in combination with a large amount of 22:1 fatty acids in chylomicrons or VLDL.

An inhibition of hormone-sensitive lipase (G in Fig. 1) could explain the lipidosis, but the activity of this enzyme is probably increased on rapeseed oil feeding as compared to an ordinary lab chow diet (30).

Kramer et al. (34) and Mersel, Heller, and Pinson

TABLE 1. Fatty acyl pattern of dietary and very low density lipoproteins triacylglycerols and heart lipids from rats fed rapeseed oil or partially hydrogenated marine oil.

		Rapeseed Oil Diet							Partially Hydrogenated Marine Oil Diet				
			Plasma[b] VLDL-TG		Heart[b]					Plasma VLDL-TG		Heart[b]	
Fatty Acid	Control Diet[a] Heart	Diet[b]	3 Days	21 Days	3 Days	21 Days	Diet[c]	Heart[c]	Diet[b]	3 Days	21 Days	3 Days	21 Days
14:0	2.5	0.7							6.5	3.6	1.8	1.2	2.2
16:0	28.2	2.8	15.2	21.3	7.2	10.3	2.8	6.0	16.1	24.1	26.4	20.5	23.0
16:1	3.0		2.8	1.6	0.6	1.6			6.9	10.6	10.7	3.5	5.7
18:0	4.0	1.0	2.0	3.6	3.2	3.3	1.1	5.5	7.7	4.8	4.4	11.6	7.8
18:1	31.5	16.3	35.4	33.2	31.4	31.9	4.7	12.1	12.8	42.2	42.9	33.7	41.5
18:2	24.1	17.9	16.9	16.7	10.7	13.0	17.2	12.3	2.1	2.3	3.0	8.0	2.8
18:3 + 20:0	1.4	9.2	4.3	2.9	3.0	3.1			4.9	1.6	1.4	1.9	2.0
20:1	1.1	0.5	5.5	5.7	12.6	11.0	2.5	2.4	11.5	6.0	4.7	9.8	9.6
20:4 + 22:0	1.9			2.6				5.5	5.0			0.7	
22:1		42.7	17.2	11.2	30.6	24.8	72.6	54.1	11.5	2.4	3.0	7.3	4.4

[a] Ordinary rat pellets with about 6% of the calories from fat.
[b] Data from Thomassen et al. (28). The acyl patterns are from heart TG.
[c] Data taken from or calculated from Beare-Rogers et al. (24). The acyl pattern is from total heart lipids.

(35) found little or no cleavage of trierucoylglycerol (G in Fig. 1) by the heart and suggested that this could be the possible basis for erucic acid lipidosis. This mechanism, however, does not seem feasible, as the acyl pattern of heart acylglycerol, even at the time of maximum lipidosis, is characterized by relatively less 22:1 fatty acid than in the dietary fat. Furthermore, very little 22:1 exists in the form of trierucoylglycerol. Altogether, the most plausible explanation of the acute TG accumulation is a slow oxidative breakdown of the long chain fatty acids in the mitochondria.

FORMATION OF ACTIVATED FATTY ACIDS IN TISSUES

Acyl-CoA

The rate of acyl-CoA formation (C in Fig. 1) is probably regulated mainly by the influx of free fatty acids and by the resulting cellular concentration of long-chain acyl-CoA, which exerts a feed-back inhibition on the activation of free fatty acids (36).

Several acyl-CoA synthases for the activation of fatty acids of different chain lengths are known. Short chain fatty acids with 2–10 carbon atoms are activated in the mitochondrial matrix (37). Their oxidation, therefore, is carnitine-independent. Long chain fatty acids are activated by extramitochondrial enzymes in the endoplasmatic reticulum and in the outer membrane of the mitochondria (38). Recent studies indicate that only one synthase activates fatty acids with more than 10 carbon atoms in rat liver microsomes (39). The activity of this synthase decreases with chain length (**Fig. 2**). Providing that the liver and the heart fatty acyl-CoA synthase have the same acyl specificity, the low reaction rate with 22:1 may explain why hearts perfused with erucic acid have a much higher content of free fatty acids than hearts perfused with the same concentration of palmitate (40, 41). However, the increased amounts of TG in the myocardium suggest that the formation of erucoyl-CoA is not rate-limiting in the metabolism of erucic acid in the heart.

Acylcarnitines

The acyl-CoA formed in cells is distributed between TG formation and acylcarnitine formation, dependent on the relative activities of the glycerophosphate acyltransferase (D in Fig. 1) (42), and the outer carnitine palmitoyltransferase (E in Fig. 1) (43, 44).

The activity of the glycerophosphate acyltransferase in liver decreases on fasting (45, 46), and it is influenced by the concentration of glycerophosphate in the cell (47).

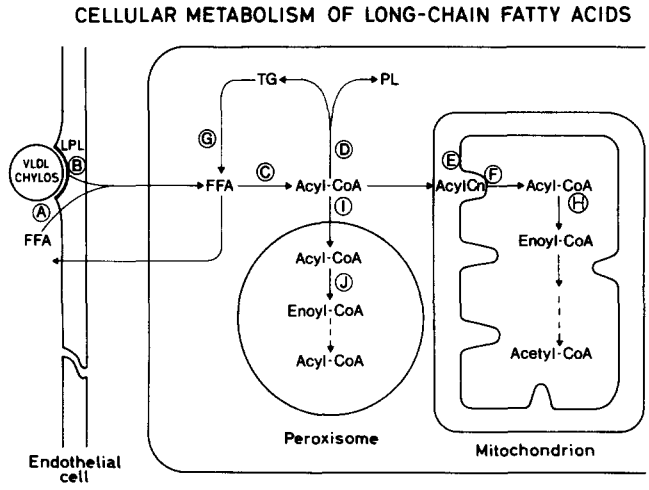

Fig. 1. The main metabolic pathways for long-chain fatty acids. The capital letters of the different reactions are referred to in the text.

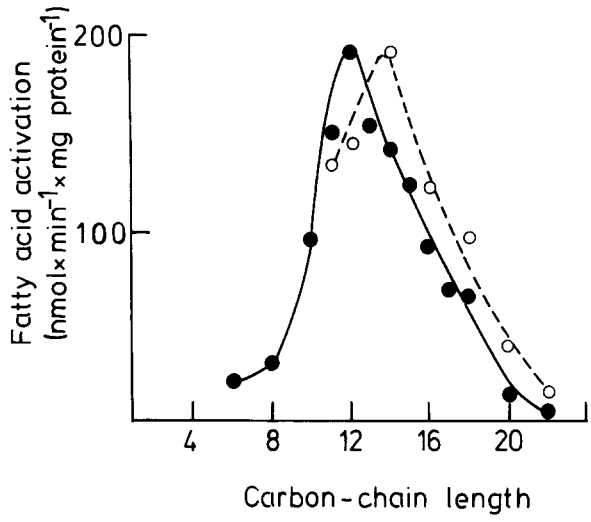

Fig. 2. Rates of activation of saturated and monounsaturated fatty acids of different chain lengths by isolated rat liver microsomes. ●, Saturated fatty acids; ○, monounsaturated fatty acids. Reproduced from reference 39 with the permission of Elsevier/North Holland Biomedical Press.

The glycerophosphate acyltransferase is much less active with erucoyl-CoA as substrate than with oleoyl-CoA (48).

The outer carnitine palmitoyltransferase of liver mitochondria increases on fasting (45, 49) and it is inhibited by malonyl-CoA (50, 51). The sensitivity of the carnitine palmityl transferase to malonyl-CoA seems to decrease on fasting (52, 53). The activity of carnitine palmitoyltransferase decreases rapidly when the fatty acid chain length increases from 18 to 22 carbon atoms (54).

The distribution of fatty acids, e.g., palmitate, between esterification and oxidation in the liver is influenced by hormones. In isolated hepatocytes, glucagon inhibits esterification and stimulates oxidation and, concomitantly, the cellular content of both long-chain acyl-CoA and long-chain acylcarnitine increases (55). This shift from esterification to oxidation seems to be the result of a decrease in both glycerophosphate concentration, thus inhibiting esterification (47), and in malonyl-CoA concentration (56), relieving the inhibition of carnitine palmitoyltransferase (51).

In the heart at least some of these regulatory mechanisms are missing. The activity of carnitine palmitoyltransferase does not depend on fasting and feeding (45), and there is probably very little malonyl-CoA since the heart has very little fatty acid synthesis.

The metabolism of erucic acid in isolated hepatocytes is almost unaffected by glucagon (57), but its oxidation is still carnitine-dependent since it is inhibited by (+)decanoylcarnitine (58). The unresponsiveness of erucic acid oxidation to glucagon is most likely connected with the need to chain-shorten erucoyl-CoA as an initial step in its metabolism (see below).

METABOLIC SHORTENING OF FATTY ACIDS

Long chain fatty acids with 18 or more carbons are known to be shortened in the intact animal, while fatty acids with 16 or less C-atoms seem to be completely oxidized once the oxidation has started. Chain-shortening has been shown for stearate (59), for polyunsaturated fatty acids with 20 and 22 carbon atoms (60), and for erucic acid (22:1) which has been recovered as oleic acid (18:1) in cultured heart cells (61) and as eicosenoic (20:1) and oleic acid in rat tissues (62). The liver seemed to be the most active organ in the conversion of erucic acid to oleic acid in the intact animal (63). When labeled erucic acid is given in *small* amounts to rats in vivo it is as rapidly oxidized to CO_2 as oleate (64). On the other hand, greater amounts of erucic acid (rapeseed oil) given in the diet evidently overload limited capacities for erucic acid shortening and oxidation, since a temporary lipidosis of the heart develops.

OXIDATION OF 22:1 FATTY ACIDS

Oxidation of 22:1 acylcarnitine in mitochondria

Acylcarnitines with fatty acid chain lengths ranging from 8 to 20 carbon atoms, both saturated and unsaturated, are good substrates for mitochondrial β-oxidation (**Fig. 3**). The inner carnitine acyltransferase (F in Fig. 1) is not rate-limiting in the oxidation of added acylcarnitines in isolated rat liver and heart mitochondria. This conclusion is based upon the observed accumulation of long-chain acyl-CoA, including erucoyl-CoA, in the mitochondria incubated with long-chain acylcarnitines (54).

There may be some differences in the ability of heart mitochondria from different mammalian species to oxidize carnitine esters of fatty acids of varying chain length. Thus, Buddecke et al. (65) found that pig heart mitochondria oxidized erucic acid with higher rates than rat heart mitochondria. These differences may be due to differences in the acyl-CoA dehydrogenases (H in Fig. 1) of the mitochondria. Furthermore, Osmundsen and Bremer (66) have shown that 22:1 fatty acids may inhibit the tricarboxylic acid cycle in heart mitochondria from the rat but not from the pig. However, it is a general feature that the oxidation rate drops abruptly when the chain length increases from 18 to 20 carbon atoms. The oxidation rate of 22:1 acylcarnitines is 30–35% of that of palmitoyl- or oleoylcarnitine (Fig. 3) (48, 54, 66). Heart and liver mitochondria from the rat show about the same relative rates (54). Even liver mitochondria from the seal *Phoca vitullina* show this slow oxidation of erucoylcarnitine (67). This seal lives in arctic waters and presumably eats a diet high in 22:1 fatty acid, because both heart and blubber lipids contain significant amounts of 20:1 and 22:1 fatty acids (68). Thus it is

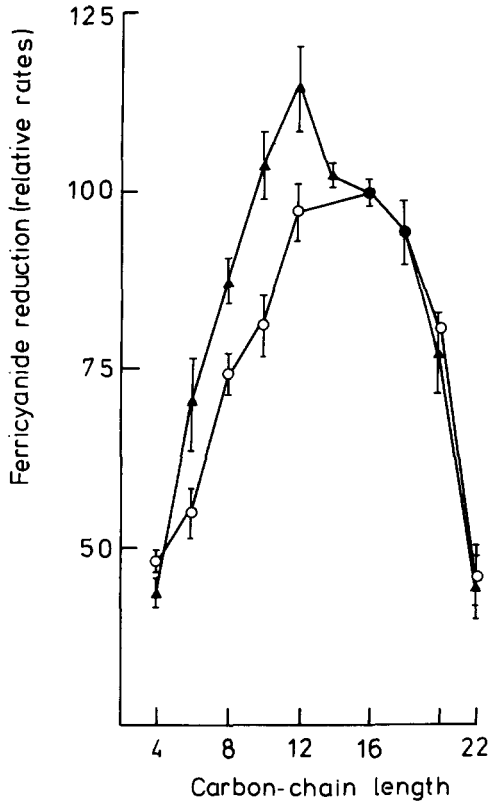

Fig. 3. β-Oxidation "spectra" of ox and pig heart mitochondria. The rates of oxidation of various saturated acylcarnitines with carbon-chain lengths from 4 to 22 carbon atoms were measured spectrophotometrically. The plotted data represent the mean relative rates of oxidation (the rate given by palmitoylcarnitine being taken as 100%) (±S.D.) based on measurements on mitochondria from two ox hearts (▲) and two pig hearts (○). The data points shown at C_{22} represent results obtained with cetoleoylcarnitine (*cis*-22:1 Δ11). Reproduced from reference 66 with the permission of the Biochemical Society.

likely that it is a general feature of mitochondria that they oxidize 22:1 acylcarnitines with a reduced rate compared to shorter chain acylcarnitines.

The rate limiting step in the oxidation of the 22:1 acylcarnitines seems to be the primary acyl-CoA dehydrogenase step (H in Fig. 1). This conclusion is based on the observation that mitochondria incubated with 22:1 acylcarnitines accumulate long-chain acyl-CoA, although at a reduced rate, compared to mitochondria incubated with palmitoylcarnitine (69). In accordance with this observation, Heijkensköld and Ernster (70) have found that a partially purified acyl-CoA dehydrogenase oxidizes erucoyl-CoA with a reduced rate compared with palmitoyl-CoA, but both substrates seemed to have the same K_m (approximately 5 μM under the assay conditions used).

Effect of 22:1 acylcarnitines on the oxidation of palmitoylcarnitine in the mitochondria

The 22:1 acylcarnitines suppress to some extent the oxidation of shorter chain acylcarnitines in the mitochondria (54). **Table 2** shows that both respiration and palmitoylcarnitine oxidation in the mitochondria are inhibited in the presence of both palmitoylcarnitine and erucoylcarnitine when compared with the rates obtained with palmitoylcarnitine alone.

Other experiments showed that total acetyl-CoA formation is also inhibited. Thus, the inhibited palmitate oxidation is only partially compensated for by the oxidation of the 22:1 fatty acids.

In liver mitochondria, cetoleoylcarnitine (22:1 *cis* Δ11), erucoylcarnitine (22:1 *cis* Δ13), and brassidoylcarnitine (22:1 *trans* Δ13) are all oxidized at similar rates. However, brassidoylcarnitine with a *trans* double bond is less inhibitory on palmitoylcarnitine oxidation. Thus, the nature of the double bond(s) may influence the metabolic effects of the 22:1 fatty acids.

Oxidation of free 22:1 fatty acids in mitochondria

Like other free fatty acids, the 22:1 fatty acids are oxidized by isolated liver mitochondria in the presence of ATP, CoA, and carnitine. Under these conditions the oxidation of erucate compared to palmitate is even slower than is the oxidation of erucoylcarnitine compared to palmitoylcarnitine (71, 72), and no inhibition of palmitate oxidation by erucate is observed. These differences from the observation with the corresponding acylcarnitines are probably explained by the observation that both the acyl-CoA synthase (39) and the carnitine palmitoyltransferase (69) show much lower activities with erucate and erucoyl-CoA, respectively, than with palmitate and palmitoyl-CoA. Erucoylcarnitine, therefore, will accumulate only slowly in such an in vitro system, and no inhibition of palmitate oxidation will occur in short term experiments.

Oxidation of 22:1 acyl-CoA in peroxisomes

Besides the "classical" β-oxidation system of mitochondria, it has been established recently that liver peroxisomes contain a different, cyanide-insensitive β-oxidation system (73, 74). The β-oxidation enzymes of peroxisomes (J in Fig. 1) are distinctly different from those of mitochondria. The peroxisomal "acyl-CoA dehydrogenase" is an acyl-CoA oxidase, a flavoprotein using oxygen as the electron acceptor with hydrogen peroxide as the reaction product (75). The enoyl-CoA hydratase, the β-hydroxyacyl-CoA dehydrogenase, and the thiolase of peroxisomes are also different from the corresponding mitochondrial enzymes, although their reaction mechanisms seem to be the same as those of the mitochondrial enzymes (76, 78).

The peroxisomes contain carnitine acetyltransferase and carnitine medium-chain acyltransferase, but carnitine palmitoyltransferase is absent (79). In accordance with this finding, the peroxisomal oxidation of fatty acid

TABLE 2. Effects of different acylcarnitines on the oxidation of [U-^{14}C]palmitoylcarnitine in the heart mitochondria

Additions	Rate of Oxygen Uptake		Disappearance of [U-^{14}C]Palmitoylcarnitine	
	ng-atoms O × min^{-1} × mg protein^{-1}	% of Inhibition (mean ± S.D.)	nmol × min^{-1} × mg protein^{-1}	% of Inhibition (mean ± S.D.)
None	238		6.5	
Erucoylcarnitine	129	45.5 ± 7.3	2.3	65.0 ± 5.5
Cetoleoylcarnitine	134	43.4 ± 6.2	2.5	62.2 ± 4.4
Brassidoylcarnitine	187	20.1 ± 2.2	4.1	37.3 ± 2.2
Gadoleoylcarnitine	224	7.7 ± 2.9	1.4	78.5 ± 3.1
Oleoylcarnitine	238	0	1.3	80.2 ± 0.7

Reproduced from reference 54 with the permission of Elsevier/North Holland Biomedical Press.

The incubation medium contained 10 mM N-tris(hydroxymethyl)methyl-2-aminoethane sulfonic acid buffer (pH 7.4), 1 mM phosphate buffer (pH 7.4), 0.1 mM 2,4-dinitrophenol, 2 mM malate, 0.5% (w/v) bovine serum albumin, 5 mM $MgCl_2$, 0.12 M KCl. The incubation volume was 3.2 ml and the temperature was 25°C. Nonlabeled acylcarnitines (100 nmol) were added after the temperature equilibration for 2.5 min. [U-^{14}C]Palmitoylcarnitine (40 nmol, approx. 3,000,000 cpm) was added 30 sec after. The reaction was stopped by addition of perchloric acid after approximately equal amounts of oxygen have been consumed from the moment of [U-^{14}C]palmitoylcarnitine addition. 1.0–1.5 mg of mitochondrial protein was used. The relative rates of the oxidation expressed as % (mean ± S.D.) of oxygen uptake with palmitoylcarnitine were: 36.6 ± 1.9, 39.3 ± 2.5, 37.0 ± 4.2, and 80.3 ± 3.8, respectively, for erucoyl-, cetoleoyl- brassidoyl-, and gadoleoylcarnitine.

esters of CoA is independent of carnitine. The peroxisomal β-oxidation system in rat liver is induced by intake of 22:1 fatty acid-containing diets (80, 81), by fasting (82), by fat feeding (80, 83), and by clofibrate and similar drugs, and by di(ethylhexylphthalate) (73, 76).

Fig. 4 shows the chain-length specificity of solubilized peroxisomes, from livers of rats treated with clofibrate, in the oxidation of CoA esters measured as the rate of NADH formation (84). The rate of NADH formation seems to be a reliable measure for the chain-length specificity of the primary acyl-CoA oxidase inasmuch as the subsequent β-oxidation enzymes (the hydratase, the β-hydroxyacyl-CoA dehydrogenase, and the thiolase) are present in excess (74).

The chain-length specificity of the peroxisomal system in the oxidation of acyl-CoA is to some extent different from that of the mitochondria measured with acylcarnitines. The main difference is that the peroxisomes are unable to oxidize short chain fatty acids (4–6 carbon atoms). With very long-chain fatty acids there is, as in mitochondria, a rapid drop in activity as the chain length increases beyond 18 carbon atoms, especially with saturated fatty acids.

The 20:1 and 22:1 fatty acyl-CoA esters are much more active as substrates for isolated peroxisomes than are the corresponding saturated ones. The significance of this in vitro difference is uncertain since the long, saturated acyl-CoA esters are less soluble than the corresponding unsaturated ones.

In accordance with the absence of a short chain acyl-CoA oxidase in the peroxisomes, complete β-oxidation of the fatty acids does not occur. Both in solubilized and in intact peroxisomes only one or a few β-oxidation cycles take place. From erucoyl-CoA, mainly 20:1 and 18:1 fatty acids are formed and from palmitoyl-CoA, mainly 14:0 and 12:0 fatty acids are formed (84). Thus, the intact peroxisomal β-oxidation is distinctly different from the mitochondrial system which forms only minute amounts of intermediate length fatty acids when long chain fatty acids are oxidized (85, 86).

The intact peroxisomal membrane seems to contain an acyl-CoA permease (I in Fig. 1) that is inhibited by free CoA (87, 88). The inhibitory effects of free CoA on this permease appear to be relatively stronger with palmitoyl-CoA than with erucoyl-CoA as substrate. Thus, it is possible that peroxisomes oxidize 22:1 acyl-CoA more selectively than is apparent from the chain length specificity curve (Fig. 4) (87).

Most of the studies performed on peroxisomal β-oxidation have so far been done with organelles isolated from liver. We know, however, that several other organs contain (micro) peroxisomes, and that homogenate fractions from these tissues (brown adipose tissue (89), heart and intestinal mucosa[2]) have a capacity for cyanide-insensitive β-oxidation. This makes it probable that these tissues have a peroxisomal β-oxidation similar to that in the liver.

ESTERIFICATION OF 22:1 FATTY ACIDS

Liver glycerophosphate acyltransferase shows a low activity with erucoyl-CoA in vitro (48), and it has been

[2] Norseth, J., and M. S. Thomassen. Personal communication.

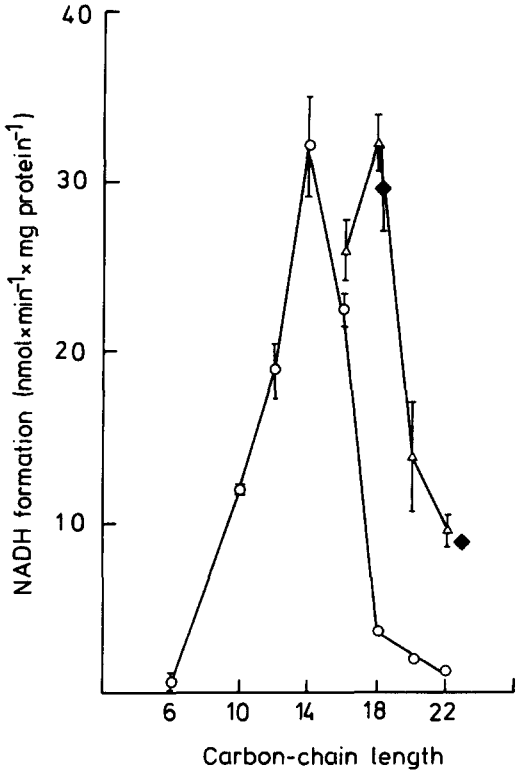

Fig. 4. Rates of oxidation of saturated and monounsaturated acyl-CoA esters of different chain lengths by isolated liver peroxisomes from rats treated with clofibrate (0.3%) in the diet for 10 days. Rates of acyl-CoA-dependent NAD reduction have been plotted against carbon chain length of the various acyl-CoA esters. The plotted values represent the mean and SD obtained with at least three different peroxisomal preparations. Results obtained with saturated acyl-CoA esters are indicated by (○) and those obtained with monounsaturated species by (△). The monounsaturated acyl-CoA esters used were: palmitoleoyl-CoA (16:1), oleoyl-CoA (18:1), gadeoyl-CoA (20:1), and erucoyl-CoA (22:1). Results obtained with the *trans*-isomers elaidoyl-CoA (18:1) and brassidoyl-CoA (22:1) are indicated by (◆). Reproduced from reference 84 with the permission of Elsevier/North Holland Biomedical Press.

shown that erucic acid is incorporated into the major phospholipids only to a small extent in rat heart (25, 26, 90).

However, Blomstrand and Svensson (91) found that heart cardiolipin contained 12% erucic acid after feeding a diet with 10% erucic acid for 10 days. Dewailly et al. (92) confirmed the cardiolipin data in principle and also found that the heart sphingomyelin contained a considerable amount of 22:1 fatty acids upon long-term feeding with rapeseed oil.

It is not known to what extent erucoyl-CoA and erucoylcarnitine accumulate in the tissues of these animals, but, in short-term feeding experiments with rapeseed oil, a higher than normal content of long-chain acyl-carnitines is found in the liver (but not in the heart) (93). In isolated cells incubated with erucic acid, erucoylcarnitine accumulates (94).

These different studies show that there is an almost general discrimination against erucic acid (and probably other 22:1 fatty acids) by the fatty acid-metabolizing enzymes in animal tissues. However, the observation that TG containing both erucic acid and more usual fatty acids accumulates in the heart and other tissues of animals fed high doses of rapeseed oil, suggests that the slow oxidation of 22:1 fatty acids and their inhibition of the oxidation of other fatty acids "force" more fatty acids into the formation of TG. This is more prominent in the heart TG of animals fed for a few days with 22:1 fatty acids, which contains more 22:1 acyl groups than liver and adipose tissue (4).

When the metabolism of erucate and palmitate is compared in normal hepatocytes and perfused normal rat hearts, the low capacity for erucic acid metabolism is confirmed (**Tables 3** and **4**). In normal hepatocytes, uptake, oxidation, and esterification of erucic acid are only about one-third of those obtained with palmitate. In the perfused heart, the oxidation of erucate is about one-third that of palmitate. However, in this organ esterification of erucate and palmitate are about equal, so that uptake of erucate in the heart is about two-thirds that of palmitate.

In isolated adipocytes, erucate is esterified with a rate one-fourth that of palmitate.[3]

ADAPTATION TO 22:1 FATTY ACIDS IN THE DIET

Adaptive changes in the heart

The decrease of the TG level in the heart after prolonged feeding of rapeseed oil may be due to adaptive changes in the heart itself or be due to altered delivery of acyl groups to the heart.

Most studies on isolated heart mitochondria from rats fed rapeseed oil for several weeks have not shown any greater ability than control mitochondria to oxidize 22:1 fatty acids (6, 34, 95, 96). Forsyth, Carter, and Loew (97) noted, however, a slightly increased capacity to oxidize palmitoyl-CoA in heart mitochondria after feeding rats with herring oil for several weeks, and Norseth et al. (40, 98) have recently reported that chain-shortening of erucic acid was increased in perfused hearts from rats fed rapeseed oil or partially hydrogenated marine oil. They also found a moderate increase of cytochrome oxidase activity, suggesting some increase in mitochondrial oxidative capacity. The increased chain-shortening could be explained by an increased peroxisomal activity in the heart. The changes are, however, not pronounced and alone can hardly explain the adaptive decrease of the TG level in the heart.

[3] Christophersen, B. O., and J. Norseth. Personal communication.

TABLE 3. Distribution of radioactivity from [14-^{14}C]erucic acid in fatty acids of liver perfusate VLDL-TG of rats fed different fat diets (30% of the calories) for 3 days or 3 weeks[a]

	Rapeseed Oil		Partially Hydrogenated Marine Oil		Groundnut Oil	
Fatty acid	3 Days	3 Weeks	3 Days	3 Weeks	3 Days	3 Weeks
16:1	8.0 ± 3.0	12.5 ± 1.4**	4.9 ± 4.1	6.5 ± 2.1	1.3 ± 1.5	1.3 ± 1.8
18:1	40.6 ± 13.9	62.3 ± 5.9**	34.8 ± 4.8	75.6 ± 9.0****	33.9 ± 7.6	34.9 ± 6.3
20:1	5.2 ± 4.0	3.8 ± 3.0	7.4 ± 2.7	3.7 ± 2.0**	5.0 ± 1.8	3.2 ± 3.1
22:1	46.2 ± 16.7	21.5 ± 3.8**	52.9 ± 5.6	14.2 ± 11.0****	59.8 ± 8.8	60.6 ± 7.0
Chain-shortened fatty acids[b]	53.8 ± 16.8	78.5 ± 3.8**	47.1 ± 5.6	85.8 ± 11.1***	40.2 ± 8.8	39.4 ± 15.4

[a] The results are presented as % of total radioactivity (cpm) + S.D. in VLDL-TG (four or five animals in each group).
[b] Sum of % of total radioactivity in 16:1, 18:1, and 20:1.
Significantly different from 3-day group: **, $0.025 > P > 0.01$; ***, $0.01 > P > 0.005$; ****, $P < 0.005$ (t-distribution).
Data are from reference 101 with the permission of the American Oil Chemists' Society.

An increased activity of the hormone-sensitive lipase (G in Fig. 1) associated with the lysosomal fraction has been found in the heart of rats fed rapeseed oil or trierucoylglycerol (99). This may be an effect of increased substrate for the lipase. Perfusion studies on hearts from these rats have shown that free erucic acid can be secreted from the heart. This secretion in the heart may be of importance in combination with the adaptation changes in the liver.

Adaptive changes in the liver

Gumpen and Norum (93) fed rats rapeseed oil and used corn oil as a control diet. They examined the relative amounts of long-chain acylcarnitine in liver and heart after feeding periods of 4 days and 4 weeks. The long-chain acylcarnitine level in the hearts of rats fed the two diets did not differ. The relative amounts of long-chain acylcarnitine in the liver, however, were significantly higher on a rapeseed than on a corn oil diet at 4 days, but not after 4 weeks, suggesting that a hepatic adaptation had taken place. The most likely adaptive change in the liver that could decrease the lipid accumulation of the heart is a change in the lipids exported from the liver and taken up by the heart. Thomassen et al. (28) therefore examined the acyl pattern in the TG of plasma VLDL in rats fed different fat diets (rapeseed oil, partially hydrogenated marine oil, or groundnut oil). The diets were fed for 3 days or for 3 weeks to reveal adaptive

TABLE 4. The metabolism of [14-^{14}C]erucic acid and of [U-^{14}C]palmitic acid in hepatocytes isolated from rats fed diets containing hydrogenated fish oil or peanut oil (control), or a standard pellet diet (2.1% fat) with and without clofibrate[a]

	Experiment I[b] (1 mM Fatty Acid)		Experiment II[c] (0.5 mM Fatty Acid)	
	Control (Peanut oil)	Hydrogenated Fish Oil	Control	Clofibrate
Erucic acid				
Oxidized	32 ± 2	93 ± 6*	16 ± 2	83 ± 8*
Phospholipids	8 ± 0.4	18 ± 2**	8 ± 2	15 ± 2**
Triacylglycerol	10 ± 1	9 ± 2	14 ± 2	22 ± 4***
Total uptake	55 ± 3	123 ± 8*	38 ± 4	121 ± 9*
Palmitic acid				
Oxidized	73 ± 3	85 ± 3***	43 ± 4	67 ± 7*
Phospholipids	22 ± 2	25 ± 2	25 ± 2	28 ± 2*
Triacylglycerol	34 ± 2	28 ± 2	43 ± 5	21 ± 4*
Total uptake	136 ± 6	143 ± 2	112 ± 4	121 ± 2***

[a] The results are given as nmol fatty acid metabolized × mg protein × 60 min^{-1} ± S.D. (four animals in each group).
[b] In experiment I, the rats (60 g initial weight) were fed the fat diets (30% of the calories) for 3 weeks.
[c] In experiment II, the rats (160–180 g) were fed a standard pellet diet with and without clofibrate (0.3%) for 8 days.
Different from control: *, $P < 0.001$; **, $P < 0.01$; ***, $P < 0.02$. Data from references 57 (Table III) and 102 (Table I) with the permission of Elsevier/North Holland Biomedical Press.

changes. The plasma VLDL-TG contained substantially less 22:1 than the diet, both after 3 days and after 3 weeks on either a rapeseed oil or a partially hydrogenated fish oil diet (Table 1). Roquelin et al. (100) also have studied plasma lipids in rats fed rapeseed oils. They found that the acyl pattern of plasma TG contained relatively less 22:1 and more 18:1 than the dietary lipids. Thomassen et al. (28) found that, in rats fed rapeseed oil, there was a decrease in 22:1 in the plasma VLDL-TG with the longer feeding time.

The acyl pattern of TG secreted during perfusion of livers from these animals also changed between 3 days and 3 weeks. There was a pronounced decrease in secretion of 22:1 acyl groups, and, concomitantly, an increased secretion of 18:1 acyl groups, suggesting that an increased chain-shortening in adapted animals had taken place. The TG remaining in the liver after the perfusion contained very small amounts of 22:1 fatty acids, suggesting that most of the 22:1 fatty acids reaching the liver are either secreted or chain-shortened (28).

Christiansen et al. (101) showed that the diet had an influence on the chain-shortening of [14-^{14}C]erucic acid in perfused liver from rats fed diets containing partially hydrogenated fish oil or rapeseed oil for 3 days or for 3 weeks. Control rats were given groundnut oil. Table 3 is taken from their publication and shows that chain-shortened products of erucic acid, mainly oleic acid, were found in all dietary groups. In the rapeseed oil and especially in the fish oil group, the chain-shortened fatty acids in TG exported from the liver increased significantly upon prolonged feeding of fats containing 22:1 fatty acids.

Table 4 shows that feeding rats diets high in hydrogenated fish oil for 2–3 weeks increases the capacity of isolated hepatocytes to metabolize erucic acid. The oxidation increased 3-fold and esterification about 1.5-fold (102). The total uptake of erucic acid in the adapted hepatocytes is nearly the same as that of palmitate (with 0.5–1 mM fatty acid in the medium). No corresponding increased uptake has been found in the heart of adapted animals (**Table 5**). Here the total uptake of erucate remains about 2/3 that of palmitate, but a paradoxical shift from oxidation to esterification was observed.

It is striking that feeding of the drug clofibrate, which is known to increase peroxisomal fatty acid oxidation (73), produces a similar and even stronger effect on erucate metabolism in hepatocytes than does feeding hydrogenated fish oil (57) (Table 4). In the heart, clofibrate increases the oxidation and uptake of erucate (41) (Table 5).

Clofibrate also gives a partial protection against heart lipidosis after feeding rapeseed oil (103). Isolated hepatocytes both from adapted rats and from rats fed clofibrate show an increased capacity to shorten erucic acid (57, 102). It is likely, therefore, that the increased rates of oxidation and esterification of erucic acid are secondary to the increased rate of chain-shortening.

An increased shortening of erucic acid is found also in perfused hearts from rats fed clofibrate or diets high in 22:1 fatty acids (Table 5). However, in the heart, the total capacity for shortening seems to be relatively much smaller than in the liver (40, 41). In the whole animal, the liver therefore seems to dominate (63).

CELLULAR SITE OF CHAIN SHORTENING

The shortening of erucic acid is evidently extramitochondrial since it apparently is stimulated by (+)decanoylcarnitine, an inhibitor of the mitochondrial carnitine palmityltransferase (**Table 6**).

The development of the increased capacity for chain shortening and oxidation of erucic acid in the liver of rats fed diets high in 22:1 fatty acids coincides in time with the development of an increased peroxisomal capacity for fatty acid oxidation (81).

Incubation of isolated peroxisomes with erucoyl-CoA leads to the formation of the same shortened products as can be isolated from hepatocytes (84). There is good reason therefore to conclude that the shortening of erucic acid can take place in the peroxisomes, both in the liver and in other tissues.

Peroxisomes may also have a role in the shortening of the side chain of cholesterol in the formation of bile acids (104).

Isolated peroxisomes shorten palmitate as well. Still, no shortened products of palmitate are found in isolated hepatocytes. However, fatty acids shorter than 16 carbon atoms are discriminated against in the biosynthesis of phospholipids and triacylglycerol (105).

Palmitate oxidation is essentially unaffected by (+)decanoylcarnitine in hepatocytes from clofibrate-fed rats (94). Since clofibrate also induces an increased activity of a medium chain acyl-CoA hydrolase in the cytoplasm of the liver (106), the shortened products of palmitate oxidized in the peroxisomes may therefore bypass the carnitine-dependent transport into the mitochondria as do externally added short chain fatty acids.

Partial β-oxidation of fatty acids, i.e., chain-shortening, has been reported to take place in an isolated microsomal fraction (107) in particle-free supernatant of the liver (108) and in the outer membrane of mitochondria (109). However, in all these studies, contamination of the different subcellular fractions with the peroxisomal β-oxidizing system was not excluded.

TABLE 5. The metabolism of [14-^{14}C]erucic acid and [U-^{14}C]palmitic acid in perfused hearts from rats fed diets containing hydrogenated fish oil or peanut oil (control), or a standard pellet diet (2.3% fat) with or without clofibrate[a]

Fatty acid	Experiment I[b]		Experiment II[c]	
	Control (Peanut oil)	Hydrogenated fish oil	Control	Clofibrate
Erucic acid (0.5 mM)				
Oxidized	715 ± 54	538 ± 125	507 ± 70	1030 ± 167*
Triacylglycerol	657 ± 62	780 ± 63**	516 ± 41	612 ± 55**
Free fatty acids	252 ± 15	256 ± 34	312 ± 16	200 ± 30*
Total uptake	1763 ± 147	1732 ± 245	1495 ± 159	2049 ± 285*
Chain-shortened	30 ± 6.5	116 ± 19*	22 ± 6	40 ± 11*
Palmitic acid (0.5 mM)				
Oxidized	2156 ± 345	1365 ± 205**	1649 ± 147	2101 ± 91*
Triacylglycerol	606 ± 68	938 ± 179**	497 ± 47	581 ± 81
Free fatty acids	25 ± 2	45 ± 2*	39 ± 10	33 ± 14
Total uptake	2953 ± 445	2612 ± 412	2379 ± 249	2924 ± 213

[a] The results are given as nmol × g heart^{-1} × 30 min^{-1} ± S.D. (five to seven animals in each group).
[b] In experiment I, the rats (60 g initial weight) were fed the fat diets (30% of the calories) for 3 weeks.
[c] In experiment II, the rats (150 g) were fed a standard pellet diet with and without clofibrate (0.3%) for 10–12 days.
Different from control: *, $P < 0.001$; **, $P < 0.01$; ***, $P < 0.02$.
Data from references 40 (Tables I and II) and 41 (Tables I and II) with the permission of Elsevier/North Holland Biomedical Press.

CHAIN-SHORTENING OF POLYUNSATURATED FATTY ACID

Oxidation and chain-shortening of long-chain polyunsaturated fatty acids represent a special problem because of the observed saturation of double bonds. Stoffel et al. (60) have suggested that shortening and saturation of double bonds in polyunsaturated fatty acids take place by partial β-oxidation and reduction in the mitochondria. The auxiliary enzyme 4-enoyl-CoA reductase, which is probably involved in this process, is a mitochondrial enzyme (110) and its activity increases both after feeding clofibrate (111) and after feeding high fat diets, especially those rich in partially hydrogenated fish oils (112). Therefore, we cannot at present exclude the possibility that the mitochondria can take part in the shortening of fatty acids, and it is still an open question as to what extent the peroxisomes are responsible for the shortening of polyunsaturated long-chain fatty acids.

However, in the metabolism of 22:1 fatty acids, the adaptive increase in the activity of the liver peroxisomal β-oxidative system seems to be the most important process, explaining the adaption to 22:1 fatty acids in the diet.

TABLE 6. The effect of carnitine and (+)-decanoylcarnitine on the metabolism of erucate in hepatocytes isolated from rats fed a diet containing hydrogenated fish oil[a]

	High Carnitine[b]		Low Carnitine[c]	
	− (+)-DC	+ (+)-DC	− (+)-DC	+ (+)-DC
Oxidized	93.7	42.1	43.4	8.2
Esterified	30.2	55.8	54.6	71.1
Uptake	123.9	97.9	97.4	79.2
Chain-shortened[d]	23.4	30.9	37.5	50.6

[a] The results are presented as mean (nmol fatty acid metabolized × mg protein^{-1} × 60 min^{-1}) from cell preparations from three rats in each group. (+)-Decanoylcarnitine ((+)-DC) was added to the concentration of 1 mM.
[b] The intracellular carnitine concentration varied from 1.8 to 2.5 nmol · mg^{-1} protein.
[c] The intracellular carnitine concentration varied from 0.25 to 0.4 nmol · mg^{-1} protein.
[d] Sum of C_{16}, C_{18}, and C_{20} radioactive fatty acids recovered from [14-^{14}C]erucic acid.
Data are from reference 58 with the permission of Elsevier/North Holland Biomedical Press.

WHAT ARE THE MECHANISMS OF ADAPTATION?

The experimental data discussed in previous paragraphs indicate that the adaptation of rats to a high content of 22:1 fatty acids in the diet is explained mainly by an increased capacity of the peroxisomal β-oxidation enzyme system to chain-shorten these long fatty acids. Admittedly, the peroxisomes, as the mitochondria, oxidize erucic acid at a low rate compared, for example, to palmitate under optimal conditions. However, in the intact cell, when the long-chain acyl-CoA is formed in the extramitochondrial compartment, it will have more direct access to peroxisomal β-oxidation that is carnitine-independent, than to mitochondrial β-oxidation that is carnitine-dependent. It is also possible that peroxisomes oxidize erucoyl-CoA with a certain preference since free

CoA seems to inhibit passage of palmityl-CoA through the peroxisomal membrane, while the passage of erucoyl-CoA is less inhibited (87).

It is striking that 22:1 fatty acids and clofibrate have so many common effects on fatty acid metabolism. Both increase the activity of peroxisomal β-oxidation system and chain-shortening of long chain fatty acids; both increase the activity of 4-enoyl-CoA reductase in the mitochondria; and both increase the activity of ornithine decarboxylase in the liver.[4] This may be coincidence, but it should be kept in mind that clofibrate is converted to a glucuronic acid conjugate (113), and it is likely, therefore, that clofibric acid is activated to a CoA ester prior to that conversion. A common feature of clofibrate and 22:1 fatty acids, therefore, may be conversion to CoA esters that are difficult to metabolize. This could represent a triggering mechanism for the adaptation. In this connection it is striking that the peroxisomal β-oxidation system increases its activity in fat feeding, fasting, and cold adaption, conditions with elevated levels of long-chain acyl-CoA in the tissues (80–83, 89).

The increased activity of ornithine decarboxylase indicates that enzyme induction (protein synthesis) mechanisms are involved in the adaptation (114).

In some respects there are also important differences in the cellular responses in rat liver to clofibrate and 22:1 fatty acids (partially hydrogenated marine oil). Recent studies using analytical differential centrifugation and morphometric analysis revealed that clofibrate induced a marked polydispersity of the peroxisomes and considerable increase in the average size of these organelles (115). However, after marine oil feeding, the size of peroxisom decreased by 10% and the particles formed a homogenous population (116). It thus appears that clofibrate stimulates peroxisomal biogenesis, while partially hydrogenated marine oil induces a more selective increase in peroxisomal β-oxidation activity.

We do not know why hydrogenated fish oil is more efficient as an adaptation inducer than is rapeseed oil with the same content of 22:1 fatty acid. The hydrogenated fish oil contains many *trans* fatty acids, and some of them are more slowly oxidized in the mitochondria than the corresponding *cis* acids (81, 117). It is possible, therefore, that some *trans* fatty acids may fortify the effect of the 22:1 fatty acids. Interference with the metabolism of the essential polyunsaturated fatty acids is also a possibility. The induction of 4-enoyl-CoA reductase (111, 112), which is an auxiliary enzyme in the metabolism of unsaturated fatty acids, is interesting in this connection. These questions are currently under active study in our laboratories.

We are grateful to our colleagues E. N. Christiansen, B. O. Christophersen, J. Norseth, H. Osmundsen, and M. S. Thomassen for fruitful collaboration. The research from our laboratories described in this article was supported by grants from the Norwegian Council for Scientific and Industrial Research and the Norwegian Research Council for Science and the Humanities.

Manuscript received 6 April 1981 and in revised form 1 September 1981.

[4] Eliassen, K., and H. Osmundsen. Personal communication.

REFERENCES

1. Abdellatif, A. M. M., and R. O. Vles. 1970. Pathological effects of dietary rapeseed oil in rats. *Nutr. Metab.* **12:** 285–295.
2. Roine, P., E. Uksila, H. Teir, and J. Rapola. 1960. Histopathological changes in rats and pigs fed rapeseed oil. *Z. Ernährungswiss.* **1:** 118–124.
3. Vles, R. O. 1975. Nutritional aspects of rapeseed oil. *In* The Role of Fats in Human Nutrition. A. J. Vergroesen, editor. Academic Press, London, PA. 433–477.
4. Beare-Rogers, J. L. 1977. Docosenoic acids in dietary fats. *Prog. Chem. Fats Other Lipids.* **15:** 29–56.
5. Ziemlanski, S. 1977. Pathophysiological effects of long-chain fatty acids. *Bibl. Nutr. Dieta.* **25:** 134–157.
6. Saurer, F. D., and J. K. G. Kramer. 1980. The metabolism of long-chain monoenoic fatty acids in heart muscle and their cardiopathologenic implications. *Adv. Nutr. Res.* **3:** 207–230.
7. Sebedio, J-L., M. F. Langman, C. A. Eaton, and R. G. Ackman. 1981. Alteration of long chain fatty acids of herring oil during hydrogenation on nickel catalyst. *J. Am. Oil Chem. Soc.* **58:** 41–48.
8. FAO Food and Nutrition Paper No. 3. 1978. Dietary Fats and Oils in Human Nutrition. Report of an Expert Committee. FAO, Rome. 1–94.
9. Thomasson, H. J. 1956. The biological value of oil and fats. IV. *J. Nutr.* **59:** 343–352.
10. Deuel, H. J., L. Hallman, and A. Leonard. 1940. The comparative rate of absorption of some natural fats. *J. Nutr.* **20:** 215–228.
11. Beare, J. L., T. K. Murray, and J. A. Campbell. 1960. Responses of two strains of rats to rapeseed oil and corn oil. *Can. J. Biochem. Physiol.* **38:** 187–192.
12. Carroll, K. K. 1957. Rapeseed oil and cholesterol metabolism in different species with reference to experimental atherosclerosis. *Proc. Soc. Exp. Biol. Med.* **94:** 202–205.
13. Walker, D. M., and G. B. Stokes. 1970. The nutritive value of fat in the diet of milkfed lamb. 1. The apparent and corrected digestibilities of different dietary fats and of their constituent fatty acids. *Br. J. Nutr.* **24:** 425–433.
14. Crampton, E. W., R. K. Shaw, V. G. Mackay, and D. C. Schad. 1960. Comparative feeding value of common edible fats as measured by the growth of prematurely weaned pups, guinea pigs and swine. *J. Nutr.* **70:** 81–90.
15. Sell, J. L., and J. A. McKirdy. 1963. Lipids in chick urine: the influence of dietary rapeseed oil. *Poult. Sci.* **42:** 380–383.
16. Paloheimo, L., and B. Jahkola. 1959. Digestibility of soybean oil and rape oil by swine. *J. Sci. Agric. Soc. Finl.* **31:** 212–214.
17. Deuel, H. J., R. M. Johnson, C. E. Calbert, J. Garner, and B. Thomas. 1949. Studies on the comparative nutri-

tive value of fats. XII. The digestibility of rapeseed and cottonseed oil in human subjects. *J. Nutr.* **38:** 369–379.
18. Hornstra, G. 1972. Digestibility, efficiency and other metabolic effects of dietary rapeseed oil in rats. *Nutr. Metab.* **14:** 282–297.
19. Caselli, C., H. Carlier, and J. Bezard. 1979. Size of lipoprotein particles in the intestinal lymph of rats fed on corn oil, peanut oil, rapeseed oil or canbra oil. *Nutr. Metab.* **23:** 73–87.
20. Novikoff, P. M., and A. B. Novikoff. 1972. Peroxisomes in absorptive cells of mammalian small intestine. *J. Cell Biol.* **53:** 532–560.
21. Fernandes, J., J. H. Van de Kamer, and H. A. Weijers. 1955. The absorption of fats studied in a child with chylothorax. *J. Clin. Invest.* **34:** 1026–1036.
22. Kramer, J. K. G., D. W. Friend, and H. W. Hulan. 1975. Lipid changes in tissues of young boars fed rapeseed oil or corn oil. *Nutr. Metab.* **19:** 279–290.
23. Opstvedt, J., H. Svaar, P. Hansen, J. Pettersen, F. T. Langmark, S. M. Barlow, and I. F. Duthie. 1979. Comparison of lipid status in the hearts of piglets and rats on short-term feeding of marine oil and rapeseed oils. *Lipids.* **14:** 356–371.
24. Beare-Rogers, J. L., E. A. Nera, and B. M. Craig. 1972. Accumulation of cardiac fatty acids in rats fed synthesized oils containing C_{22} fatty acids. *Lipids.* **7:** 46–50.
25. Houtsmuller, U. M. T., C. B. Struijk, and A. Van der Beek. 1970. Decrease in rate of ATP synthesis of isolated rat heart mitochondria induced by dietary erucic acid. *Biochim. Biophys. Acta.* **218:** 564–566.
26. Dow-Walsh, D. S., S. Mahadevan, J. K. G. Kramer, and F. D. Sauer. 1975. Failure of dietary erucic acid to impair oxidative capacity or ATP production of rat heart mitochondria isolated under controlled conditions. *Biochim. Biophys. Acta.* **396:** 125–132.
27. Ziemlanski, S., A. Rosnowski, and T. Opuszynska-Freyer. 1973. Ultrastructure of early fatty infiltration in the myocardium after administration of rapeseed oil in the diet. *Acta Med. Pol.* **14:** 279–290.
28. Thomassen, M. S., E. Strøm, E. N. Christiansen, and K. R. Norum. 1979. Effect of marine oil and rapeseed oil on composition of fatty acids in lipoprotein triacylglycerols from rat blood plasma and liver perfusate. *Lipids.* **14:** 58–65.
29. Shafrir, E., S. Gatt, and S. Khasis. 1965. Partition of fatty acids of 20–24 carbon atoms between serum albumin and lipoproteins. *Biochim. Biophys. Acta.* **98:** 365–371.
30. Hülsmann, W. C., M. M. Geelhoed-Mieras, H. Jansen, and U. M. T. Houtsmuller. 1979. Alteration of the lipase activities of muscle, adipose tissue and liver by rapeseed oil feeding of rats. *Biochim. Biophys. Acta.* **572:** 183–187.
31. Kramer, J. K. G., and H. W. Hulan. 1978. The effect of dietary erucic acid on cardiac triglycerides and free fatty acid levels in rats. *Lipids.* **13:** 438–445.
32. Jansen, H., W. C. Hülsmann, A. van Zuylen-van Wiggen, C. B. Struijk, and U. M. T. Houtsmuller. 1975. Influence of rapeseed oil feeding on the lipase activities of rat heart. *Biochem. Biophys. Res. Commun.* **64:** 747–751.
33. Hülsmann, W. C., and H. Stam. 1978. Intracellular origin of hormone-sensitive lipolysis in the rat. *Biochem. Biophys. Res. Commun.* **82:** 53–59.
34. Kramer, J. K. G., S. Mahadevan, J. R. Hunt, F. D. Sauer, A. H. Corner, and K. M. Charlton. 1973. Growth rate, lipid composition, metabolism and myocardial lesions of rats fed rapeseed oils (*Brassica campestris* var. Arlo, Echo and Span, and *B. napus* var. Oro). *J. Nutr.* **103:** 1696–1708.
35. Mersel, M., M. Heller, and A. Pinson. 1979. Intracellular lipase activities in heart and skeletal muscle homogenates. The absence of trierucin cleavage by the heart: a possible biochemical basis for erucic acid lipidosis. *Biochim. Biophys. Acta.* **572:** 218–224.
36. Oram, J. F., J. I. Wenger, and J. R. Neely. 1975. Regulation of long chain fatty acid activation in heart muscle. *J. Biol. Chem.* **250:** 73–78.
37. Aas, M. 1971. Organ and subcellular distribution of fatty acid activating enzymes in the rat. *Biochim. Biophys. Acta.* **231:** 32–47.
38. Norum, K. R., M. Farstad, and J. Bremer. 1966. The submitochondrial distribution of acid:CoA ligase (AMP) and palmityl-CoA:carnitine palmityltransferase in rat liver mitochondria. *Biochem. Biophys. Res. Commun.* **24:** 797–804.
39. Normann, P. T., M. S. Thomassen, E. N. Christiansen, and T. Flatmark. 1981. Acyl-CoA synthetase activity of rat liver microsomes. Substrate specificity with special reference to very long-chain and isomeric fatty acids. *Biochim. Biophys. Acta.* **664:** 416–427.
40. Norseth, J. 1979. The effect of feeding rats with partially hydrogenated marine oil or rapeseed oil on the chain-shortening of erucic acid in perfused heart. *Biochim. Biophys. Acta.* **575:** 1–9.
41. Norseth, J. 1980. Increased β-oxidation of erucic acid in perfused hearts from rats fed clofibrate. *Biochim. Biophys. Acta.* **617:** 183–191.
42. Daae. L. N. W., and J. Bremer. 1970. The acylation of glycerophosphate in rat liver. A new assay procedure for glycerophosphate acylation, studies on its subcellular and submitochondrial localization and determination of the reaction products. *Biochim. Biophys. Acta.* **210:** 92–104.
43. Norum, K. R., and J. Bremer. 1967. The localization of acyl coenzyme A-carnitine acyltransferase in rat liver cells. *J. Biol. Chem.* **242:** 407–411.
44. Hoppel, C. L., and R. J. Tomec. 1972. Carnitine palmityltransferase. Location of two enzymatic activities in rat liver mitochondria. *J. Biol. Chem.* **247:** 832–841.
45. Aas, M., and L. N. W. Daae. 1971. Fatty acid activation and acyl transfer in organs from rats in different nutritional states. *Biochim. Biophys. Acta.* **239:** 208–216.
46. Borrebæk, B. 1975. Acylation of carnitine and glycerophosphate in suspensions of rat liver mitochondria at varying levels of palmitate and coenzyme A. *Acta Physiol. Scand.* **95:** 448–456.
47. Lund, H., B. Borrebæk, and J. Bremer. 1980. Regulation of palmitate esterification/oxidation by glycagon in isolated hepatocytes. The role of α-glycerophosphate concentration. *Biochim. Biophys. Acta.* **620:** 364–371.
48. Christophersen, B. O., and J. Bremer. 1972. Erucic acid—an inhibitor of fatty acid oxidation in the heart. *Biochim. Biophys. Acta.* **280:** 506–514.
49. Norum, K. R. 1965. Activation of palmityl-CoA:carnitine palmityltransferase in livers from fasted, fat-fed, or diabetic rats. *Biochim. Biophys. Acta.* **98:** 652–654.
50. McGarry, J. D., and D. W. Foster. 1979. In support of the roles of malonyl-CoA and carnitine acyltransferase I in the regulation of hepatic fatty acid oxidation and ketogenesis. *J. Biol. Chem.* **254:** 8163–8168.

51. McGarry, J. D., and D. W. Foster. 1980. Regulation of hepatic fatty acid oxidation and ketone body production. *Ann. Rev. Biochem.* **49:** 395–420.
52. Cook, G. A., D. A. Otto, and N. W. Cornell. 1980. Differential inhibition of ketogenesis by malonyl-CoA in mitochondria from fed and starved rats. *Biochem. J.* **192:** 955–958.
53. Ontko, J. A., and M. L. Johns. 1980. Evaluation of malonyl-CoA in the regulation of long-chain fatty acid oxidation in the liver. *Biochem. J.* **192:** 959–962.
54. Christiansen, R. Z., B. O. Christophersen, and J. Bremer. 1977. Monoethylenic C_{20} and C_{22} fatty acids in marine oil and rapeseed oil. Studies on their oxidation and on their relative ability to inhibit palmitate oxidation in heart and liver mitochondria. *Biochim. Biophys. Acta.* **487:** 28–36.
55. Christiansen, R. Z. 1977. Regulation of palmitate metabolism by carnitine and glucagon in hepatocytes isolated from fasted and carbohydrate-refed rats. *Biochim. Biophys. Acta.* **488:** 249–262.
56. Cook, G. A., R. C. Nielsen, R. A. Hawkins, M. A. Mehlman, M. R. Lakshaman, and R. L. Veech. 1977. Effect of glucagon on hepatic malonyl coenzyme A concentration and on lipid synthesis. *J. Biol. Chem.* **252:** 4421–4424.
57. Christiansen, R. Z. 1978. The effect of clofibrate-feeding on hepatic fatty acid metabolism. *Biochim. Biophys. Acta.* **530:** 314–324.
58. Christiansen, R. Z., E. N. Christiansen, and J. Bremer. 1979. The stimulation of erucate metabolism in isolated rat hepatocytes by rapeseed oil and hydrogenated marine oil-containing diets. *Biochim. Biophys. Acta.* **573:** 417–429.
59. Elovson, J. 1965. Chain-shortening of stearic acid in the intact rat. *Biochim. Biophys. Acta.* **98:** 36–40.
60. Stoffel, W., W. Ecker, H. Assad, and H. Sprecher. 1970. Enzymatic studies on the mechanism of the retroconversion of C_{22}-polyenoic fatty acids to their C_{20}-homologues. *Hoppe-Seyler's Z. Physiol. Chem.* **351:** 1545–1554.
61. Pinson, A., and P. Padieu. 1974. Erucic acid oxidation by beating heart cells in culture. *FEBS Lett.* **39:** 88–90.
62. Craig, B. M., and J. L. Beare. 1967. The β-oxidative degradation of docosenoic acids to eicosenoic and octadecenoic acids in the rat. *Can. J. Biochem.* **45:** 1075–1079.
63. Ong, J., J. Bexard, and J. Lecerf. 1977. Incorporation and metabolic conversion of erucic acid in various tissues of the rat in short term experiments. *Lipid.* **12:** 563–569.
64. Carroll, K. K. 1962. Levels of radioactivity in tissues and in expired carbon dioxide after administration of 1-C^{14}-labeled palmitic acid, 2-C^{14}-labeled erucic acid, or -C^{14}-labeled nervonic acid to rats. *Can. J. Biochem. Physiol.* **40:** 1229–1238.
65. Buddecke, von E., I. Filipovic, B. Wortberg, and A. Seher. 1976. Wirkungsmechanismus langkettiger Monoenfettsäuren im Energistoffwechsel des Herzens. *Fette Seifen Anstrichm.* **5:** 196–200.
66. Osmundsen, H., and J. Bremer. 1978. Comparative biochemistry of β-oxidation. An investigation into the abilities of isolated heart mitochondria of various animal species to oxidize long-chain fatty acids, including the $C_{22:1}$ monoenes. *Biochem. J.* **174:** 379–386.
67. Osmundsen, H. 1981. Spectrophotometric procedure for measuring mitochondrial β-oxidation. *Methods Enzymol.* **72:** 306–314.
68. Ackman, R. G., and S. N. Hooper. 1974. Long-chain monoethylenic and other fatty acids in heart, liver, and blubber lipids of two harbor seals (*Phoca vitulina*) and one grey seal (*Halichoerus grypus*). *J. Fish. Res. Board Can.* **31:** 333–341.
69. Christophersen, B. O., and R. Z. Christiansen. 1975. Studies on the mechanism of the inhibitory effects of erucylcarnitine in rat heart mitochondria. *Biochim. Biophys. Acta.* **388:** 402–412.
70. Heijkenskjøld, L., and L. Ernster. 1975. Studies of the mode of action of erucic acid on heart metabolism. *Acta Med. Scand. Suppl.* **585:** 75–83.
71. Swarttouw, M. A. 1974. The oxidation of erucic acid by rat heart mitochondria. *Biochim. Biophys. Acta.* **337:** 13–21.
72. Christiansen, E. N., and E. J. Davis. 1978. The effects of coenzyme A and carnitine on steady-state ATP/ADP ratios and the rate of long-chain free fatty acid oxidation in liver mitochondria. *Biochim. Biophys. Acta.* **502:** 17–28.
73. Lazarow, P. B., and C. de Duve. 1976. A fatty acyl-CoA oxidation system in rat liver peroxisomes; enhancement by clofibrate, a hypolipidemic drug. *Proc. Natl. Acad. Sci. USA.* **73:** 2043–2046.
74. Lazarow, P. B. 1978. Rat liver peroxisomes catalyze the β-oxidation of fatty acids. *J. Biol. Chem.* **253:** 1522–1528.
75. Osumi, T., T. Hashimoto, and N. Ul. 1980. Purification and properties of acyl-CoA oxidase from rat liver. *J. Biochem.* **87:** 1735–1746.
76. Osumi, T., and T. Hashimoto. 1979. Subcellular distribution of the enzymes of the fatty acyl-CoA β-oxidation system and their induction by di(2-ethylhexyl)phthalate in rat liver. *J. Biochem.* **85:** 131–139.
77. Furuta, S., S. Miyazawa, T. Osumi, T. Hashimoto, and N. Ul. 1980. Properties of mitochondrial and peroxisomal enoyl-CoA hydratases from rat liver. *J. Biochem.* **88:** 1059–1070.
78. Miyazawa, S., T. Osumi, and T. Hashimoto. 1980. The presence of a new 3-oxoacyl-CoA thiolase in rat liver peroxisomes. *Eur. J. Biochem.* **103:** 589–596.
79. Markwell, M. A. K., L. L. Bieber, and N. E. Tolberg. 1977. Differential increase of hepatic peroxisomal, mitochondrial and microsomal carnitine acyltransferases in clofibrate-fed rats. *Biochem. Pharmacol.* **26:** 1697–1702.
80. Neat, C. E., M. S. Thomassen, and H. Osmundsen. 1980. Induction of peroxisomal β-oxidation in rat liver by high-fat diets. *Biochem. J.* **186:** 369–371.
81. Neat, C. E., M. S. Thomassen, and H. Osmundsen. 1981. Effects of high-fat diets on hepatic fatty acid oxidation in the rat. Isolation of rat liver peroxisomes by vertical-rotor centrifugation by using a self-generated, iso-osmotic, Percoll gradient. *Biochem. J.* **196:** 149–159.
82. Ishil, H., S. Horie, and T. Suga. 1980. Physiological role of peroxisomal β-oxidation in liver of fasted rats. *J. Biochem.* **87:** 1855–1858.
83. Ishi, H., N. Fukumori, S. Horie, and T. Suga. 1980. Effects of fat content in the diet on hepatic peroxisomes of the rat. *Biochim. Biophys. Acta.* **617:** 1–11.
84. Osmundsen, H., C. E. Neat, and K. R. Norum. 1979. Peroxisomal oxidation of long chain fatty acids. *FEBS Lett.* **99:** 292–296.
85. Stanley, K. K., and P. K. Tubbs. 1975. The role of intermediates in mitochondrial fatty acid oxidation. *Biochem. J.* **150:** 77–88.
86. Lopes-Cardozo, M., W. Klazinga, and S. G. van den

Bergh. 1978. Accumulation of carnitine esters of β-oxidation intermediates during palmitate oxidation by rat-liver mitochondria. *Eur. J. Biochem.* **83:** 629–634.
87. Osmundsen, H., and C. E. Neat. 1979. Regulation of peroxisomal fatty acid oxidation. *FEBS Lett.* **107:** 81–85.
88. Appelkvist, E. L., and G. Dallner. 1980. Possible involvement of fatty acid binding protein in peroxisomal β-oxidation of fatty acids. *Biochim. Biophys. Acta.* **617:** 156–160.
89. Nedergaard, J., S. Alexon, and B. Cannon. 1980. Cold adaptation in the rat: increased brown fat peroxisomal β-oxidation relative to maximal mitochondrial oxidative capacity. *Am. J. Physiol.* **239:** C208–C216.
90. Blomstrand, R., and L. Svensson. 1974. Studies on phospholipids with particular reference to cardiolipin of rat heart after feeding rapeseed oil. *Lipids.* **9:** 771–780.
91. Blomstrand, R., and L. Svensson. 1975. Observations on lipid composition with particular reference to cardiolipin of rat heart after feeding rapeseed oil. *Acta Med. Scand.* Suppl. **585:** 51–73.
92. Dewailly, P., G. Sezille, A. Nouvelot, J. C. Fruchart, and J. Jaillard. 1977. Changes in rat heart phospholipid composition after rapeseed oil feeding. *Lipids.* **12:** 301–306.
93. Gumpen, S. A., and K. R. Norum. 1973. The relative amounts of long-chain acylcarnitines, short-chain acylcarnitines and carnitine in heart, liver and brown adipose tissue from rats fed on rapeseed oil. *Biochim. Biophys. Acta.* **316:** 48–55.
94. Osmundsen, H., R. Z. Christiansen, and J. Bremer. 1980. Extra-mitochondrial shortening of long chain fatty acids and carnitine-dependent fatty acid oxidation. *In* Carnitine Biosynthesis, Metabolism, and Functions. R. A. Frenkel and J. M. McGarry, editors. Academic Press, New York. 127–142.
95. Galli Kienle, M., and G. Cighetti. 1976. Effects of rapeseed oil on fatty acid oxidation and lipid levels in rat heart and liver. *Lipids.* **11:** 670–675.
96. Ming Lee Hsu, C., and F. A. Kummerow. 1977. Influence of elaidate and erucate on heart mitochondria. *Lipids.* **12:** 486–494.
97. Forsyth, G. W., K. E. Carter, and F. M. Loew. 1977. Heart mitochondrial metabolism after feeding herring oil to rats and monkeys. *Lipids.* **12:** 791–796.
98. Norseth, J., E. N. Christiansen, and B. O. Christophersen. 1979. Increased chain shortening of erucic acid in perfused heart from rats fed rapeseed oil. *FEBS Lett.* **97:** 163–165.
99. Stam, H., T. Geelhoed-Mieras, and W. C. Hülsmann. 1980. Erucic acid-induced alteration of cardiac triglyceride hydrolysis. *Lipids.* **15:** 242–250.
100. Rocquelin, G., P. O. Astorg, J. C. Peleran, and P. Juanceda. 1974. Influence des huiles de colza et de canbra sur les lipides plasmatiques du rat. *Nutr. Metab.* **16:** 305–316.
101. Christiansen, E. N., M. S. Thomassen, R. Z. Christiansen, H. Osmundsen, and K. R. Norum. 1979. Metabolism of erucic acid in perfused rat liver: increased chain shortening after feeding partially hydrogenated marine oil and rapeseed oil. *Lipids.* **14:** 829–835.
102. Christiansen, R. Z., E. N. Christiansen, and J. Bremer. 1979. The stimulation of erucate metabolism in isolated rat hepatocytes by rapeseed oil and hydrogenated marine oil-containing diets. *Biochim. Biophys. Acta.* **573:** 417–429.
103. Christiansen, R. Z., J. Norseth, and E. N. Christiansen. 1979. The effect of clofibrate on heart and plasma lipids in rats fed a diet containing rapeseed oil. *Lipids.* **14:** 614–618.
104. Pedersen, J. I., and J. Gustafsson. 1980. Conversion of 3α,7α,12α-trihydroxy-5β-cholestanoic acid into cholic acid by rat liver peroxisomes. *FEBS Lett.* **121:** 345–348.
105. Kornberg, A., and W. E. Pricer, Jr. 1953. Enzymatic esterification of α-glycerophosphate by long chain fatty acids. *J. Biol. Chem.* **204:** 345–357.
106. Borrebæk, B., H. Osmundsen, and J. Bremer. 1979. Increased levels of hepatic acyl-CoA hydrolase activities in clofibrate fed rats. *IRCS Med. Sci.: Biochem.* **7:** 181.
107. Chang, H-C., and R. T. Holman. 1972. Chain shortening of acyl-coenzyme A by rat liver microsomes. *Biochim. Biophys. Acta.* **280:** 17–21.
108. Fiecchi, A., M. Galli-Kienle, A. Scala, G. Galli, and R. Paoletti. 1973. The β-oxidative cleavage of long-chain fatty acids in rat-liver cytoplasm. *Eur. J. Biochem.* **38:** 516–528.
109. Clouet, P., and J. Bezard. 1978. Chain shortening of erucic acid by subcellular particles isolated from liver and heart of rat. *FEBS Lett.* **93:** 165–168.
110. Kunau, W-H., and P. Dommes. 1978. Degradation of unsaturated fatty acids. Identification of intermediates in the degradation of *cis*-4-decenoyl-CoA by extracts of beef liver mitochondria. *Eur. J. Biochem.* **91:** 533–544.
111. Borrebæk, B., H. Osmundsen, and J. Bremer. 1980. In vivo induction of 4-enoyl-CoA reductase by clofibrate in liver mitochondria and its effect on pent-4-enoate metabolism. *Biochem. Biophys. Res. Commun.* **93:** 1173–1180.
112. Borrebæk, B., H. Osmundsen, E. N. Christiansen, and J. Bremer. 1980. Increased 4-enoyl-CoA reductase activity in liver mitochondria of rats fed high-fat diets and its effect on fatty acid oxidation and the inhibitory action of pent-4-enoate. *FEBS Lett.* **121:** 23–24.
113. Cayen, M. N., E. S. Ferdinandi, E. Greselin, W. T. Robinson, and D. Dvornik. 1977. Clofibrate and clofibraic acid: comparison of the metabolic disposition in rats and dogs. *J. Pharm. Exp. Therap.* **200:** 33–43.
114. Jänne, J., H. Pösö, and A. Raina. 1978. Polyamines in rapid growth and cancer. *Biochim. Biophys. Acta.* **473:** 241–293.
115. Flatmark, T., E. N. Christiansen, and H. Kryvi. 1981. Polydispersity of rat liver peroxisomes induced by the hypolipidemic and carcinogenic agent clofibrate. *Eur. J. Cell Biol.* **24:** 62–69.
116. Christiansen, E. N., T. Flatmark, and H. Kryvi. 1981. Effects of marine oil diet on peroxisomes and mitochondria of rat liver. A combined biochemical and morphometric study. *Eur. J. Cell Biol.* **26:** 11–20.
117. Lawson, L. D., and F. A. Kummerow. 1979. β-Oxidation of the coenzyme A esters of elaidic, oleic, and stearic acids and their full-cycle intermediates by rat heart mitochondria. *Biochim. Biophys. Acta.* **573:** 245–254.