

Peroxisomes in liver, heart, and kidney of mice fed a commercial fish oil preparation: original data and review on peroxisomal changes induced by high-fat diets

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Abstract Male NMRI mice were fed a diet with 10% w/w Beromegan[®] for up to three weeks. Beromegan[®] is a commercial fish (salmon) oil preparation rich in eicosapentaenoic acid and docosahexaenoic acid. Peroxisomal β -oxidation capacity, catalase activity, and ultrastructural morphometry of the hepatic peroxisomes were investigated. In myocardium and kidney, catalase activity, peroxisomal staining after catalase cytochemistry, peroxisomal morphology, and morphometry (in myocardium) were evaluated. In liver, we found a significant increase in peroxisomal β -oxidation, catalase activity, and peroxisomal number already after 3 days of dietary treatment. These changes were more pronounced after 3 weeks. Peroxisomal size was not changed. Positive correlations were found between peroxisomal enzyme activities and the number but not the size of the peroxisomes, and between catalase activity and β -oxidation capacity. The mean peroxisomal diameter per animal was inversely proportional to catalase activity measured in homogenate. In myocardium, catalase activity was increased with duration of fish oil feeding. Peroxisomal staining, number, and size were also increased when compared to controls. In kidney, no alterations were observed. **Our results indicate a beneficial effect of a diet supplemented with fish oil on the peroxisomal metabolism in liver and myocardium; it differs from the changes induced by xenobiotic peroxisome proliferation.—De Craemer, D., J. Vamecq, F. Roels, L. Vallée, M. Pauwels, and C. Van den Branden.** Peroxisomes in liver, heart, and kidney of mice fed a commercial fish oil preparation: original data and review on peroxisomal changes induced by high-fat diets. *J. Lipid Res.* 1994. 35: 1241–1250.

Supplementary key words catalase activity • catalase cytochemistry • morphometry • docosahexaenoic acid • polyunsaturated fatty acids • peroxisomal β -oxidation

Peroxisomes are cell organelles characterized by the presence of hydrogen peroxide-producing oxidases and high amounts of catalase which breaks down hydrogen peroxide to water and oxygen (1). Based on its abundance in peroxisomes, catalase is used as a marker enzyme in

fractionation studies and in morphologic studies using (immuno-) cytochemical techniques. Peroxisomes also contain enzymes that intervene in several metabolic processes such as the β -oxidation of very long chain fatty acids and the synthesis of plasmalogens (ether-phospholipids) and bile acids (2).

Peroxisomes had long been considered as relatively unimportant organelles until it was reported that peroxisomes were absent in the livers of patients with the cerebro-hepato-renal syndrome of Zellweger (3). Today it is known that at least 13 metabolic diseases are related to a deficiency in one or more peroxisomal enzymes (4, 5). Besides severe clinical symptoms, an accumulation of the very long chain fatty acids is always observed in patients with an inborn error of peroxisomal β -oxidation (6). A dietary therapy for patients suffering from one of these disorders (X-linked adrenoleukodystrophy) consists of a diet supplemented with a mixture of glyceryltriolate and glyceryltriolate (Lorenzo's oil) and a dietary restriction of the very long chain fatty acids (7). This results in a reduction of the level of very long chain fatty acids in the majority of treated patients. Clinical symptoms however, do not improve. Only some patients with a mild form of the disease or at the onset of the clinical symptoms showed a stabilization of the neurological impairment (7–10).

Recently, Martinez (11, 12) found that patients with generalized peroxisomal diseases revealed a constant biochemical abnormality: the content of docosahexaenoic acid, a polyunsaturated fatty acid (C22:6) of the n-3 series, was decreased in brain, liver and kidney. In a

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single published case, supplementation with the pure ethyl ester of docosahexaenoic acid resulted in a clearcut improvement (13). Following these data, investigation of polyunsaturated fatty acids in all peroxisomal deficient patients was requested (14). In a large scale trial in the United States, n-3 fatty acids in the form of fish oil are now being added to the diet of patients with X-linked adrenoleukodystrophy in order to avoid essential fatty acid deficiency in patients on Lorenzo's oil therapy (10).

Our aim was to investigate the effects of a diet supplemented with fish oil, which contains a high concentration of docosahexaenoic acid, on the peroxisomal metabolism of laboratory animals for up to 3 weeks. We used the commercial fish oil preparation (Beromegan®) in a concentration that made it possible to compare our results with those of Yamazaki, Shen, and Schade (15): the two main constituents in fish oil (eicosapentaenoic acid C20:5, n-3 and docosahexaenoic acid C22:6, n-3) reached a final concentration in the chow administered to the laboratory animals of approximately 3% and 2%, respectively.

The results showed that fish oil activates peroxisomal functions in liver and myocardium but not in kidney. Preliminary findings in the liver of mice on dietary treatment for 3 days were presented at the Joint Meeting of the Dutch and Belgian Societies for Electron Microscopy and the Belgian Society for Cell Biology (16).

MATERIAL AND METHODS

Adult male NMRI mice were randomly assigned into six groups. Five groups were fed commercial powdered chow supplemented with 10% Beromegan® (a gift from FHER, division of Boehringer Ingelheim) for 1, 2, 3, 14, or 21 days. Beromegan® is a fish (salmon) oil extract containing mainly polyunsaturated fatty acids (57%) (Table 1).

TABLE 1. Fatty acid composition of Beromegan®

Fatty Acid	Percent of Total
14:0	6
16:0	13
16:1	8
16:2	1
17:0	1
18:0	3
18:1	10
18:2	1.5
18:4	1
20:0	1
20:4	0.5
20:5	30
22:4	1
22:5	2
22:6	20

Besides fatty acids, Beromegan also contains \pm 0.025% α -tocopherol and 1% cholesterol.

The control group received standard powdered chow (A04 meal-UAR, Epinay, France). All animals had free access to food and water and were fed the standard powdered chow at least 5 days before the start of the experiment.

All mice were killed by cervical dislocation after ether anesthesia and the liver, heart, and right kidney were removed. For histological analysis, thin slices (1 mm thick) were cut from the middle lobe of the liver, from the left ventricle wall, and from the kidney cortex. The liver and kidney slices were fixed in cacodylate-buffered 4% formaldehyde for 24 h at room temperature (17). Slices of heart were fixed in the same solution for 18 h at 0°C. This modification permits an enhancement of the peroxisomal staining in tissues with relatively small amounts of catalase (I. Kerckaert and F. Roels, unpublished observation). Cryosections (50 μ m thick) of the fixed material were incubated with diaminobenzidine at pH 10.5 (catalase staining of peroxisomes) (18) and evaluated under the light microscope, or were postosmicated in the presence of $K_3Fe(CN)_6$, and embedded in epoxy resin. On 2- μ m epoxy resin sections, periportal areas in the liver and longitudinal sectioned myocardial fibers were selected for ultrastructural investigation. Morphometry of the hepatocellular and myocardial peroxisomes was performed on random electron micrographs (17).

The remaining parts of the liver were frozen to -80°C before enzyme assays were performed. Peroxisomal fatty acyl-CoA oxidase specific activity (first step of the peroxisomal β -oxidation) was studied in homogenate using the method of Vamecq (19) and is expressed in nmol hydrogen peroxide generated per minute and mg protein. Cyanide-insensitive fatty acyl-CoA oxidation was measured as described by Lazarow and de Duve (20) and is expressed as nmol NAD^+ reduced per minute and mg protein. Catalase activity was assayed at 0°C in homogenate of liver, heart, and kidney of each mouse using the titanium oxysulfate method (21, 22). One U_B is the amount of catalase that breaks down 90% of the substrate (1.5 mM $\text{H}_2\text{O}_2/1$) in a volume of 50 ml at 0°C in 1 min; maximal reaction time is 10 min.

As statistical methods (23), we used linear regression analysis and non-parametrical Kruskal-Wallis test. Significance was obtained when $P < 0.05$.

RESULTS

Relative organ weights

The diet rich in fish oil was well accepted by the mice. At the end of the experiment, body weight of Beromegan®-fed animals was similar to the weight of control animals (not shown). Hepatomegaly was observed in Beromegan®-fed animals: liver weight expressed as percentage of body weight was increased 25% after 3, 14, and 21 days of feed-

ing, when compared to animals fed the control diet. No differences in heart or kidney weights were observed.

Enzyme assays

Already after 2 and 3 days, hepatic catalase activity was significantly increased in mice fed the chow supplemented with 10% fish oil (110 ± 17 and 136 ± 10 U_B/g liver, respectively) when compared to the animals fed the standard chow (86 ± 16 U_B/g liver). In mice fed the fish oil diet for up to 3 days, a linear increase in hepatic catalase activity as a function of duration of feeding was observed (Fig. 1). A continuing significant but less pronounced increase in hepatic catalase activity was found between the third and fourteenth day of feeding (157 ± 9 U_B/g liver). Feeding the mice 1 week longer did not provoke an additional increase in catalase activity (161 ± 14 U_B/g liver) (Fig. 1).

Peroxisomal β -oxidation of fatty acyl-CoAs in the liver gradually increased with longer periods of fish oil intake. The rate-limiting step in peroxisomal β -oxidation, measured as lauroyl-CoA and palmitoyl-CoA oxidase activities, was already significantly increased after 3 days of fish oil feeding (Fig. 2). After 3 weeks of Beromegan[®]-

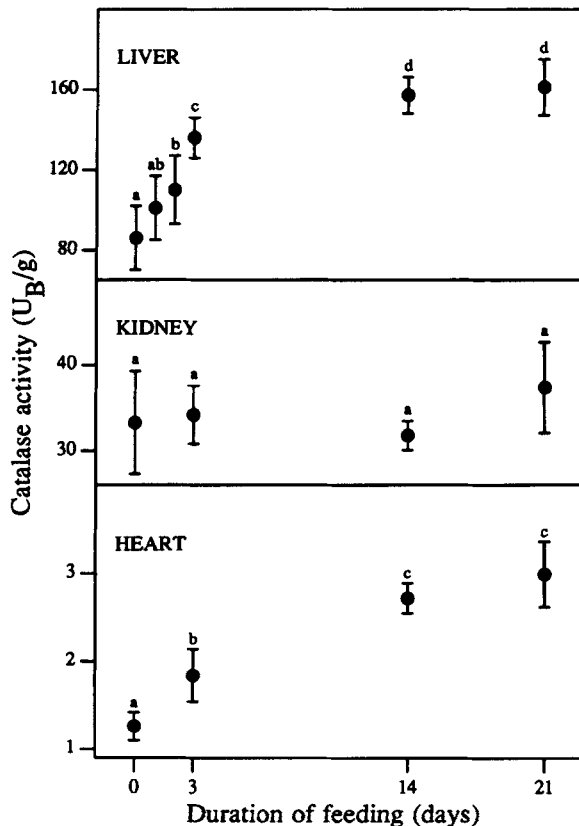


Fig. 1. Effect of duration of feeding on the catalase activity in liver, kidney, and myocardium of mice fed a diet supplemented with 10% (w/w) fish oil (Beromegan[®]). Points without a common superscript are significantly different at $P < 0.05$.

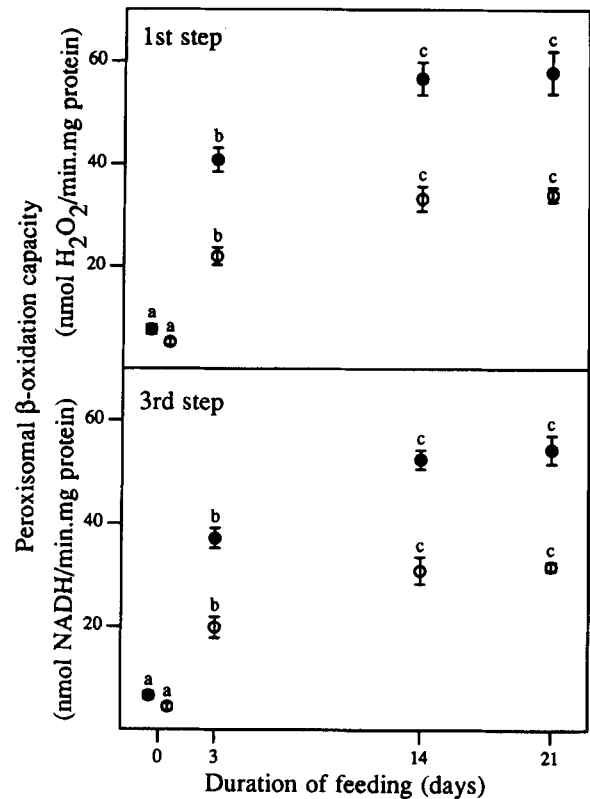


Fig. 2. Effect of duration of feeding on the first and third step of the peroxisomal β -oxidation capacity in the liver of mice fed a diet supplemented with 10% (w/w) fish oil (Beromegan[®]) using lauroyl-CoA (●) and palmitoyl-CoA (○) as substrates. Points without a common superscript are significantly different at $P < 0.05$.

supplemented diet, the activities of lauroyl-CoA oxidase and palmitoyl-CoA oxidase were increased, respectively, 7.7- and 6.6-fold when compared to the activities in control mouse livers (Fig. 2).

Hepatic peroxisomal β -oxidation capacity was also measured as the cyanide-insensitive fatty acyl-CoA-dependent NAD⁺ reduction using lauroyl-CoA and palmitoyl-CoA as substrates (third step in peroxisomal β -oxidation). The amount of NAD⁺ reduced was significantly increased as a function of duration of feeding the fish oil-supplemented diet (Fig. 2). After 3 weeks, cyanide-insensitive lauroyl-CoA and palmitoyl-CoA oxidation were increased, respectively, 8.3- and 7.2-fold. Hepatic catalase activity was positively correlated ($r > 0.90$; $P < 0.001$) with peroxisomal β -oxidation (results not shown).

Renal catalase activity in mice fed the fish oil diet for periods up to 3 weeks was never different from that in control mice (Fig. 1). Myocardial catalase activity was significantly increased after 3 days (1.82 ± 0.30 U_B/g) when compared to the activity in control myocardium (1.26 ± 0.16 U_B/g) and reached 2.99 ± 0.37 U_B/g in mice fed the fish oil-supplemented diet for 3 weeks (Fig. 1).

Morphology of peroxisomes

Light microscopic evaluation revealed peroxisomal proliferation and an increase in catalase staining of the peroxisomes in all livers of mice fed the fish oil-supplemented diet (Fig. 3). Peroxisomal size was unchanged. No accumulation of lipid droplets in the hepatocytes was observed. Ultrastructural observation of the hepatic peroxisomes revealed an increase in number in fish oil-fed animals. In all livers, peroxisomal matrix was homogeneously filled with electron-dense catalase reaction product. In all control as well as treated livers, peroxisomal shape varied widely: oval, elongated, triangular, angular, and reniform organelles were observed. Sometimes short DAB-positive tails were present. In this respect, mouse liver differs from rat and human liver.

In the myocardium of mice fed the diet with Bero-megan®, staining intensity of the peroxisomes was increased (Fig. 4). By light microscopic evaluation, peroxisomes were larger and more numerous than in the myocardial cells of control mice. Ultrastructural analysis of the myocardium revealed the presence of lipid droplets in two mice fed the fish oil-supplemented diet and in one control mouse. After catalase cytochemistry, peroxisomes were recognized by a reticular matrix in the myocardium of both control and fish oil-fed mice. They were found in the near vicinity of mitochondria and elements of sarco-

plasmic reticulum. By visual evaluation, peroxisomal number and size were increased after fish oil feeding confirming light microscopic observations.

By light microscopy, catalase staining of the renal peroxisomes did not reveal differences in staining intensity, number, or distribution of the organelles between animals fed the fish oil-supplemented versus the control diet (not shown).

Morphometry of peroxisomes

Ultrastructural morphometry of the hepatic peroxisomes confirmed the light microscopic impression: the number of peroxisomes was doubled in fish oil-fed mice for 3 days (+98%) and for 21 days (+124%). Although mean peroxisomal size was unchanged, this resulted in a significant doubling of the volume and surface density of the peroxisomal compartment. Between the third and the twenty first day of fish oil feeding, differences in peroxisomal diameter, number, volume, and surface density were no longer observed (Table 2).

A significant linear positive relationship was found between the number of peroxisomes (numerical density) on the one hand, and the catalase activity (Fig. 5), the palmitoyl-CoA oxidase activity, and the cyanide-insensitive palmitoyl-CoA oxidation on the other hand. Similar relationships were found with volume and surface density of

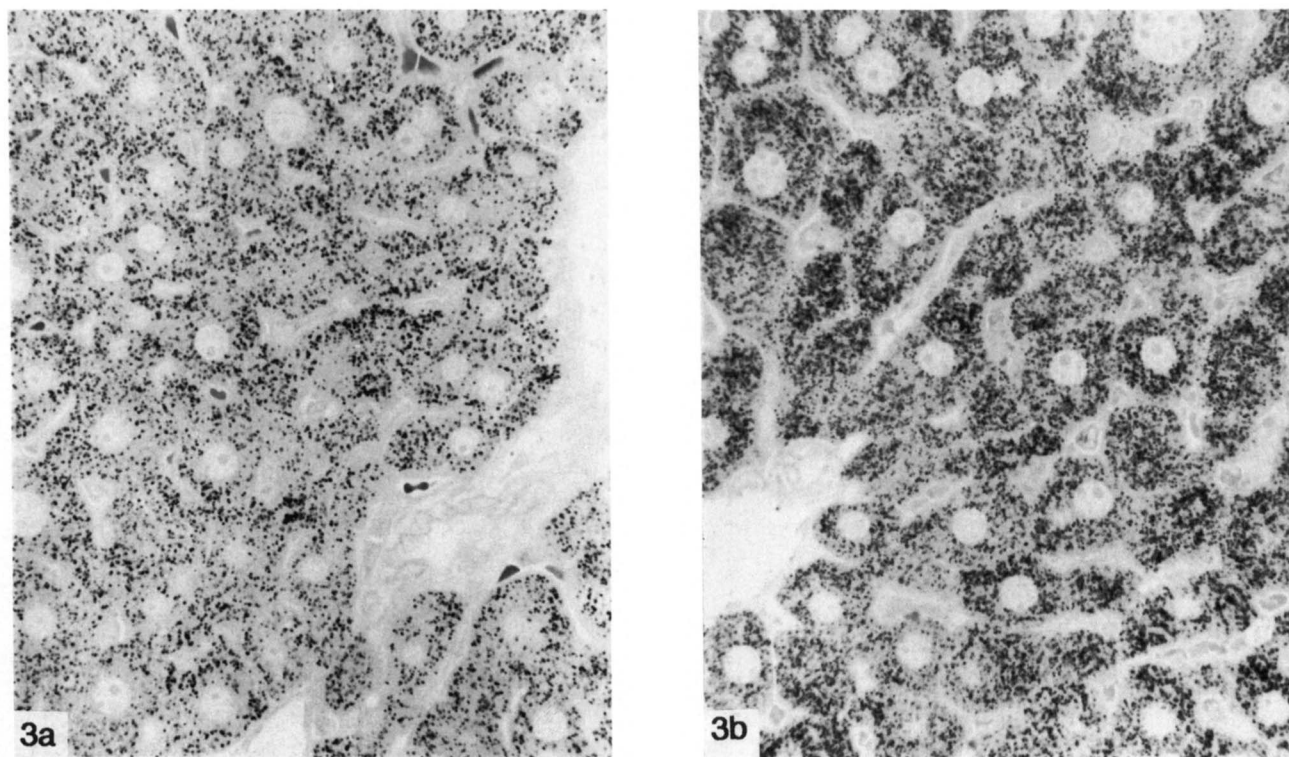


Fig. 3. Light micrographs of the liver of a mouse fed the control diet (a) and of a mouse fed the fish oil-supplemented diet for 3 days (b). Catalase stain; peroxisomes are recognized as black dots in the cytoplasm. An increase in peroxisomal number is observed in the liver of mice fed the fish oil-supplemented diet. Final magnification: $\times 500$.

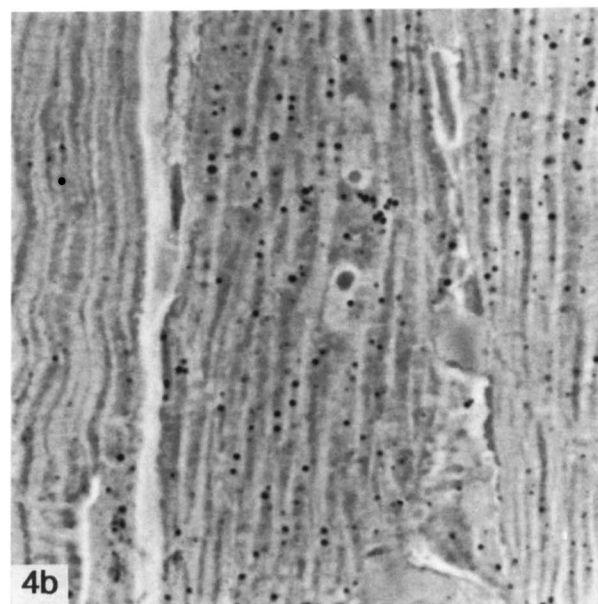
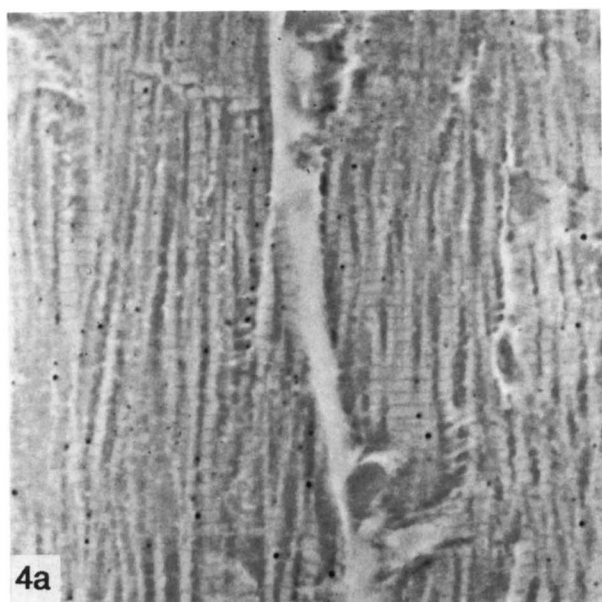


Fig. 4. Light micrographs of the myocardium of a mouse fed the control diet (a) and of a mouse fed the fish oil-supplemented diet for 14 days (b). Catalase stain; peroxisomes (black dots) are larger and more numerous in myocardium of mice fed the fish oil-supplemented diet. Final magnification: $\times 1250$.

the hepatic peroxisomes. In contrast, mean peroxisomal diameter was not related to catalase activity (**Fig. 6**) and peroxisomal β -oxidation when control and treated animals were pooled into one group. However, we did find an inverse relationship between mean peroxisomal diameter and catalase activity per animal in control mice as well as in fish oil-fed mice (**Fig. 6**). This means that smaller hepatic peroxisomes have a higher enzyme activity than larger peroxisomes. This agrees with the finding of Roels and Cornelis (24) that smaller peroxisomes have higher catalase concentrations than larger ones. It was proposed that the larger surface area to volume ratio of smaller peroxisomes is favorable for translocation of the enzyme molecules (which are synthesized in the cytoplasm), as well as for exchange of metabolites between peroxisomes and cytosol (25, 26).

With respect to peroxisomal shape, no changes in mean or median axial ratio were found between livers of

animals fed the fish oil-supplemented diet and control mice, not even when peroxisomes were divided in groups according to size.

Ultrastructural morphometry of the peroxisomes in the myocardium of mice fed the Beromegan[®]-supplemented diet for 14 days was in agreement with the light and electron microscopic impression. A 1.7-fold increase in number (numerical density) and a 1.3-fold increase in size were found when compared to control mice. This resulted in a 3.8-fold and 2.9-fold increase in volume density and surface density, respectively (**Table 3**).

DISCUSSION

Our study on mouse liver confirms previous observations in rats after a fish oil-supplemented diet for 14 days; hepatomegaly and increased activities of catalase and

TABLE 2. Morphometric analysis of hepatic peroxisomes

Diet (Animals)	Mean D-circle		Volume Density %	Numerical Density μm^{-3}	Surface Density μm^{-1}
	Measured μm	Corrected* μm			
Controls (n = 8)	0.382 ± 0.006^a	0.470 ± 0.007^a	1.641 ± 0.146^a	0.433 ± 0.037^a	0.240 ± 0.020^a
Fish oil (3 days) (n = 5)	0.390 ± 0.007^a	0.483 ± 0.010^a	3.374 ± 0.133^b	0.857 ± 0.067^b	0.482 ± 0.022^b
Fish oil (21 days) (n = 5)	0.370 ± 0.010^a	0.457 ± 0.014^a	3.299 ± 0.235^b	0.970 ± 0.058^b	0.491 ± 0.023^b

Values without a common superscript are significantly different at $P < 0.01$; *, corrected for sectioning effect; mean \pm S.E.M.

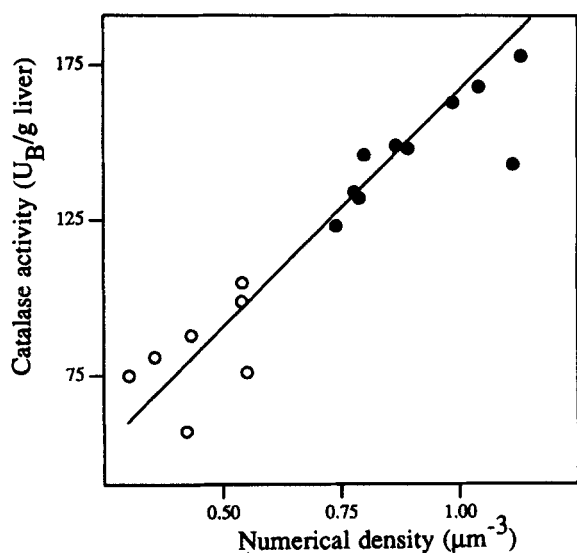


Fig. 5. Significant linear correlation between the number (numerical density) of peroxisomes (X-axis) and the catalase activity in the liver (Y-axis) of control mice (○) and mice fed the fish oil-supplemented diet (●): $Y = 27.5 + 130X$; $r = 0.94$; $P < 0.001$.

peroxisomal fatty-acyl CoA oxidase were found (15). In addition, we observed an increase of the cyanide-insensitive fatty acyl-CoA oxidation (which assay ensures a specific measurement of the peroxisomal β -oxidation), an increased staining of the peroxisomes after catalase cytochemistry, and increased numbers of peroxisomes. These hepatic changes were already evident after a short period of feeding (3 days).

An increase in peroxisomal β -oxidation evidenced by an enhancement of the capacity of either its first (hydrogen peroxide production) or third (NADH production) step in liver homogenates or in peroxisomal-enriched fractions was also reported in rodents fed a diet with high amounts of linol salad oil (27), partially hydrogenated marine (fish) oil (28–33), and fish oil (15, 34). Flatmark et al. (33) found that with a 20% (w/w) partially hydrogenated fish oil diet, a 5.3-fold increase in cyanide-insensitive β -oxidation in rodent liver coincided with a 12-fold increase of the mRNA level for the bifunctional enzyme representing an enzyme induction by this diet. Ishii et al. (27) proposed that the high fat concentration in the diet was the primary cause for the induction of the peroxisomes. However, Christiansen, Flatmark, and Kryvi (29) found no differences in palmitoyl-CoA-dependent dehydrogenase activity in the liver of rats fed a diet supplemented with different concentrations of soya-bean oil while Thomassen, Christiansen, and Norum (31) reported a gradual increase in peroxisomal β -oxidation after feeding rats a diet with increasing amounts of partially hydrogenated marine oil.

It has also been suggested that a deficiency of an essential fatty acid in some high-fat diets provoked peroxisomal

induction (29). There are two essential unsaturated fatty acids known in mammals: linoleic acid (C18:2, n-6) and α -linolenic acid (C18:3, n-3) (35). Two distinct sets of unsaturated fatty acids can be derived by desaturation and elongation of the two essential fatty acids (35, 36). In our experiment, both families were present in the diet.

Based on literature data and their own findings on the effects of dietary *trans*- and *cis*-monounsaturated and saturated fatty acids, Veerkamp and Zevenbergen (37) suggested that high-fat diets only induce peroxisomal β -oxidation when they also contain C20 and C22 fatty acids. Five years earlier, Neat, Thomassen, and Osmundsen (30) had already noticed that the most marked induction of peroxisomal β -oxidation was observed with diets containing large amounts of fatty acids that are poorly oxidized by mitochondrial β -oxidation. In this regard, increased peroxisomal β -oxidation has been linked to the presence of high amounts of monounsaturated fatty acids (C22:1) in partially hydrogenated marine (fish) oil (38). However, erucic acid (C22:1) is not present in Beromegan® but C20 and C22 fatty acids do make up 53.5% (w/w) (Table 1).

Recently, evidence was presented that eicosapentaenoic acid (C20:5, n-3) and docosahexaenoic acid (C22:6, n-3) in fish oil (these fatty acids are absent in partially hydrogenated marine (fish) oils) are of particular importance for the stimulation of the peroxisomal β -oxidation in liver. Isolated rat liver cells are able to retroconvert docosahexaenoic acid to eicosapentaenoic acid by one

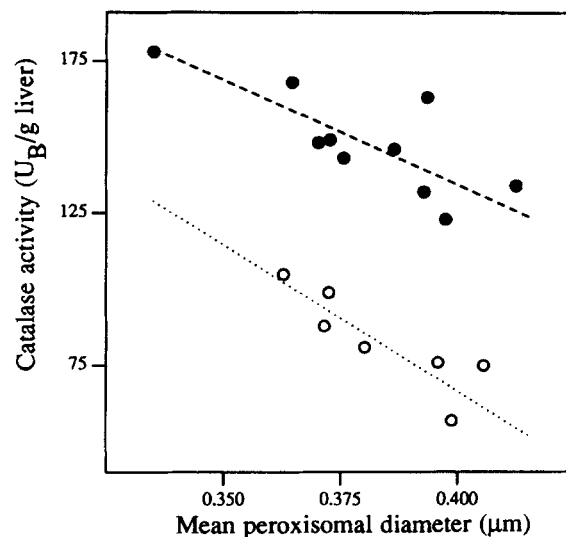


Fig. 6. Correlation between peroxisomal size (X-axis) and hepatic catalase activity (Y-axis) in mice fed the fish oil-supplemented diet (●) and in control mice (○). A significant inverse correlation was found in each group of mice: $Y = 380.654 - 0.6112X$; $r = 0.77$; $P < 0.01$ (dashed line) and $Y = 410.189 - 0.8525X$; $r = 0.86$; $P < 0.02$ (dotted line). No significant correlation was found when all mice were pooled in one group.

TABLE 3. Morphometric analysis of myocardial peroxisomes

Diet (Animals)	Mean D-circle		Volume Density	Numerical Density	Surface Density
	Measured	Corrected*			
	μm	μm	%	μm^{-3}	μm^{-1}
Controls (n = 4)	0.306 \pm 0.011 ^a	0.364 \pm 0.016 ^a	0.042 \pm 0.009 ^a	0.023 \pm 0.002 ^a	0.008 \pm 0.001 ^a
Fish oil (14 days) (n = 4)	0.395 \pm 0.013 ^b	0.476 \pm 0.017 ^b	0.161 \pm 0.023 ^b	0.039 \pm 0.005 ^b	0.023 \pm 0.003 ^b

Values without a common superscript are significantly different at $P < 0.05$; *, corrected for sectioning effect; mean \pm S.E.M.

cycle of saturation and peroxisomal β -oxidation (39, 40). Furthermore, docosahexaenoic acid may be an inhibitor of the mitochondrial β -oxidation (41) but recently this was not confirmed (42). Both polyunsaturated fatty acids, when separately added in highly purified form to a diet, provoke an increase in peroxisomal β -oxidation (42–44).

An increase in hepatic catalase activity has been previously reported in rodents fed high-fat diets containing linol salad oil (27), partially hydrogenated marine oil (28–30, 45), and fish oil (15). Catalase is a scavenger of hydrogen peroxide that is produced at the first step of each cycle of the peroxisomal β -oxidation. The linear correlation we found between catalase activity and peroxisomal β -oxidation is functionally meaningful and is an indication of a coordinated control mechanism in the induction of several different enzymes. A coordinated increase of catalase and β -oxidation capacity is not found when xenobiotic peroxisome proliferators such as clofibrate are administered. The effect of peroxisome proliferators also differs from the effect of natural fatty acids because organelle size is increased by clofibrate among others. The disproportionate increase of β -oxidation compared to catalase has been proposed as a mechanism of clofibrate-induced hepatocarcinogenesis (46).

Morphometric analysis of hepatic peroxisomes in rats fed a high partially hydrogenated fish oil diet for 25 days revealed a 1.5-fold increase in peroxisomal number (29). We already observed a doubling of the number of peroxisomes after 3 days of fish oil diet (Table 2) and this was linearly related to peroxisomal β -oxidation activation and catalase activity (Fig. 5). Peroxisomal proliferation, an induction of peroxisomal β -oxidation, and an increase in catalase activities have also been reported in liver of rodents after administration of several hypolipidemic drugs (47–49), thyroid hormones (50, 51), and inhibitors of mitochondrial β -oxidation (52), and in experimental rat diabetes (53). Size (Table 2) and shape of the hepatic peroxisomes were not altered in fish oil-fed mice. Peroxisomes are smaller in hyperthyroidism and after treatment with inhibitors of mitochondrial β -oxidation (54), and are larger under the influence of hypolipidemic drugs (54) and also in hypothyroidism (55).

It is well known that diets rich in n-3 fatty acids play a favorable role in preventing and ameliorating cardiovascular diseases (56). The effects of dietary fish oil on myocardial peroxisomes have not been described before. In heart homogenates of rats fed diets supplemented with partially hydrogenated marine oil and rapeseed oil, an induction of the peroxisomal β -oxidation was observed (57). Catalase activity was also increased in these animals (57, 58). Starvation, experimentally induced diabetes, and treatment with clofibrate or inhibitors of mitochondrial β -oxidation all provoked an increase in peroxisomal β -oxidation, catalase activity, and peroxisomal number in myocardium of rodents (52, 59–63). We observed an increase in catalase activity, peroxisomal staining, and number after dietary fish oil treatment. These peroxisomal changes are most probably related to the high amounts of docosahexaenoic acid and eicosapentaenoic acid in the fish oil diet. Both fatty acids are effectively absorbed from the intestine in rats (64). It is interesting to note that a high fat diet with a high ratio of n-3/n-6 polyunsaturated fatty acids also induces an increase in peroxisomal β -oxidation in gastric mucosa of rats when compared to diets with a low ratio (65). Docosahexaenoic acid is transported in the blood via albumin and a second, so far unidentified protein (66). Cultured rat cardiomyocytes have the ability to take up eicosapentaenoic acid and docosahexaenoic acid (67). These cells are also able to retroconvert C22-acids to C20-acids via one cycle of the peroxisomal β -oxidation (67). The produced hydrogen peroxide may be neutralized by catalase.

Renal peroxisomes are large and most numerous in the proximal tubules suggesting that these organelles fulfill a specific role in this part of the nephron segment (68, 69). In a recent review, a multiple role for the peroxisomes in the renal metabolism was suggested: it may include substrate interconversion, β -oxidation of fatty acids, and acyl compounds poorly oxidized by mitochondria, biotransformation, and detoxification of xenobiotics, ether-lipid synthesis, and in the carnivore kidney pheromone synthesis (70). Experimental evidence of peroxisomal function in vivo was reported by Van den Branden, Verbeelen, and Roels (71) who found increased hydrogen peroxide pro-

duction in the remnant kidney concomitant with the increase in Na⁺ reabsorption, which is an energy-requiring process. Possibly, peroxisomal β -oxidation accounts for the additional hydrogen peroxide (71).

To our knowledge, data on the effects of high fat diets on renal peroxisomal functions are lacking. Rodents treated with hypolipidemic drugs or fed thyroxine revealed an increase in renal peroxisomal β -oxidation enzyme and catalase activity (47, 72, 73). We observed no changes in catalase activity and peroxisomal staining in the kidneys of intact mice fed a fish oil-supplemented diet for 3 weeks. Therefore, in the kidney as well as in the liver, the effects of clofibrate and fat feeding differ.

The mechanism by which a dietary fish oil elicits an induction of the peroxisomes in liver and heart is not clear. Recently, a nuclear receptor inducible by peroxisome proliferators has been reported (74) but the natural ligand has not yet been identified. Based on recent results on diets enriched with different concentrations of fish oil and corn oil, it was speculated that an n-3 fatty acid or a molecule derived from it may induce the peroxisome proliferator activated receptor (75). Our observations indicate that a fish oil supplement in the diet of normal mice induces peroxisomes in liver and heart as did diets with partially hydrogenated marine oils. The fish oil preparation Beromegan[®] is an inexpensive diet supplement that may increase the extreme low concentrations of docosahexaenoic acid in the nervous system of a group of peroxisomal patients; this might result in an amelioration of the clinical symptoms. However, further studies on the effects of lower (therapeutic) doses of fish oils on peroxisomes of experimental animals are needed. ■

The authors wish to thank René Stien for his indispensable technical assistance. Supported by a grant of the National Fonds voor Wetenschappelijk Onderzoek (2923220790).

Manuscript received 27 September 1993 and in revised form 24 January 1994.

REFERENCES

- De Duve, C., and P. Baudhuin. 1966. Peroxisomes (microbodies and related particles). *Physiol. Rev.* **46**: 323-357.
- van den Bosch, H., R. B. H. Schutgens, R. J. A. Wanders, and J. M. Tager. 1992. Biochemistry of peroxisomes. *Annu. Rev. Biochem.* **61**: 157-197.
- Goldfisher, S., C. L. Moore, A. B. Johnson, A. J. Spiro, M. P. Valsamis, H. M. Wisniewski, R. H. Ritch, W. T. Norton, I. Rapin, and L. M. Gartner. 1973. Peroxisomal and mitochondrial defects in the cerebro-hepato-renal syndrome. *Science*. **182**: 62-64.
- Lazarow, P. B., and H. W. Moser. 1989. Disorders of peroxisome biogenesis. In *The Metabolic Basis of Inherited Disease*. C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle, editors. McGraw-Hill, New York. 1479-1509.
- Wanders, R. J. A., R. B. H. Schutgens, P. G. Barth, J. M. Tager, and H. van den Bosch. 1993. Postnatal diagnosis of peroxisomal disorders: a biochemical approach. *Biochimie*. **75**: 269-279.
- Wanders, R. J. A., C. W. T. van Roermund, R. B. H. Schutgens, P. G. Barth, H. S. A. Heymans, H. van den Bosch, and J. M. Tager. 1990. The inborn errors of peroxisomal β -oxidation: a review. *J. Inher. Metab. Dis.* **13**: 3-36.
- Rizzo, W. B., R. T. Leshner, A. Odone, A. L. Dammann, D. A. Craft, M. E. Jensen, S. S. Jennings, S. Davis, R. Jaitly, and J. A. Sgro. 1989. Dietary erucic acid therapy for X-linked adrenoleukodystrophy. *Neurology*. **39**: 1415-1422.
- Uziel, G., E. Bertini, M. Rimoldi, and M. Gambetti. 1990. Italian multicentric dietary therapeutic trial in adrenoleukodystrophy. In *Adrenoleukodystrophy and Other Peroxisomal Disorders. Clinical, Biochemical, Genetic and Therapeutic Aspects*. G. Uziel, R. J. A. Wanders, and M. Cappa, editors. Excerpta Medica, Amsterdam. 163-180.
- Moser, H. W., A. Bergin, S. Naidu, and P. W. Ladenson. 1991. Adrenoleukodystrophy. *Endocrinol. Metab. Clin. N. Am.* **20**: 297-318.
- Moser, H. W., A. B. Moser, K. D. Smith, A. Bergin, J. Borel, J. Shankroff, O. C. Stine, C. Merette, J. Ott, W. Krivit, and E. Shapiro. 1992. Adrenoleukodystrophy: phenotypic variability and implications for therapy. *J. Inher. Metab. Dis.* **15**: 645-664.
- Martinez, M. 1991. Developmental profiles of polyunsaturated fatty acids in the brain of normal infants and patients with peroxisomal diseases: severe deficiency of docosahexaenoic acid in Zellweger's and Pseudo-Zellweger's Syndromes. In *World Review of Nutrition and Dietetics*. A. P. Simopoulos, R. E. Kifer, R. E. Martin, and S. M. Barlow, editors. Karger, Basel. 87-102.
- Martinez, M. 1992. Abnormal profiles of polyunsaturated fatty acids in the brain, liver, kidney and retina of patients with peroxisomal disorders. *Brain Res.* **583**: 171-182.
- Martinez, M. 1992. Treatment with docosahexaenoic acid favorably modifies the fatty acid composition of erythrocytes in peroxisomal patients. *Prog. Clin. Biol. Res.* **375**: 389-397.
- Roels, F., S. Fischer, and W. Kissling. 1993. Polyunsaturated fatty acids in peroxisomal disorders: a hypothesis and a proposal for treatment. *J. Neurol. Neurosurg. Psychiat.* **56**: 937.
- Yamazaki, R. K., T. Shen, and G. B. Schade. 1987. A diet rich in (n-3) fatty acids increases peroxisomal β -oxidation activity and lowers plasma triacylglycerols without inhibiting glutathione-dependent detoxication activities in the rat liver. *Biochim. Biophys. Acta.* **920**: 62-67.
- De Craemer, D., F. Roels, and C. Van den Branden. 1993. Rapid effects of dietary fish oil on peroxisomes in mouse liver. *Eur. J. Morphol.* **30**: 331-335.
- De Craemer, D., I. Kerckaert, and F. Roels. 1991. Hepatocellular peroxisomes in human alcoholic and drug-induced hepatitis: a quantitative study. *Hepatology*. **14**: 811-817.
- Roels, F., A. Cornelis, B. T. Poll-The, P. Aubourg, H. Ogier, J. Scotto, and J. M. Saudubray. 1986. Hepatic peroxisomes are deficient in infantile Refsum disease: a cytochemical study of 4 cases. *Am. J. Med. Genet.* **25**: 257-271.
- Vamecq, J. 1990. Fluorometric assay of peroxisomal oxidases. *Anal. Biochem.* **186**: 340-349.
- Lazarow, P. B., and C. de Duve. 1976. A fatty acyl-CoA oxidizing system in rat liver peroxisomes: enhancement by clofibrate, a hypolipidemic drug. *Proc. Natl. Acad. Sci. USA.* **73**: 2043-2046.
- Baudhuin, P. 1974. Isolation of rat liver peroxisomes. *Methods Enzymol.* **31**: 358-368.

22. Van den Branden, C., I. Kerckaert, and F. Roels. 1984. Peroxisomal β -oxidation from endogenous substrates. Demonstration through H_2O_2 production in the unanaesthetized mouse. *Biochem. J.* **218**: 697-702.
23. Snedecor, G. W., and W. G. Cochran. 1980. Statistical Methods. Iowa State University Press, Ames, IA.
24. Roels, F., and A. Cornelis. 1989. Heterogeneity of catalase staining in human hepatocellular peroxisomes. *J. Histochem. Cytochem.* **37**: 331-337.
25. Roels, F., M. Espeel, and D. De Craemer. 1991. Liver pathology and immunocytochemistry in congenital peroxisomal diseases. *J. Inher. Metab. Dis.* **14**: 857-875.
26. Roels, F., M. Espeel, F. Poggi, H. Mandel, L. Van Maldergem, and J. M. Saudubray. 1993. Human liver pathology in peroxisomal diseases: a review including novel data. *Biochimie.* **75**: 281-292.
27. Ishii, 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.
28. 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.
29. 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.
30. Neat, C. E., M. S. Thomassen, and H. Osmundsen. 1981. Effects of high-fat diets on hepatic fatty acid oxidation in the rat. *Biochem. J.* **196**: 149-159.
31. Thomassen, M. S., E. N. Christiansen, and K. R. Norum. 1982. Characterization of the stimulatory effect of high-fat diets on peroxisomal β -oxidation in rat liver. *Biochem. J.* **206**: 195-202.
32. Thomassen, M. S., J. Norseth, and E. N. Christiansen. 1985. Long-term effects of high-fat diets on peroxisomal β -oxidation in male and female rats. *Lipids.* **20**: 668-674.
33. Flatmark, T., A. Nilsson, J. Kvannes, T. S. Eikhom, M. H. Fukami, H. Kryvi, and E. N. Christiansen. 1988. On the mechanism of induction of the enzyme systems for peroxisomal β -oxidation of fatty acids in rat liver by diets rich in partially hydrogenated fish oil. *Biochim. Biophys. Acta.* **962**: 122-130.
34. Rustan, A. C., E. N. Christiansen, and C. A. Drevon. 1992. Serum lipids, hepatic glycerolipid metabolism and peroxisomal fatty acid oxidation in rats fed ω -3 and ω -6 fatty acids. *Biochem. J.* **283**: 333-339.
35. Sardesai, V. M. 1992. Nutritional role of polyunsaturated fatty acids. *J. Nutr. Biochem.* **3**: 154-166.
36. Drevon, C. A. 1992. Marine oils and their effects. *Nutr. Rev.* **50**: 38-45.
37. Veerkamp, J. H., and J. L. Zevenbergen. 1986. Effect of dietary fat on total and peroxisomal fatty acid oxidation in rat tissues. *Biochim. Biophys. Acta.* **878**: 102-109.
38. Bremer, J., and K. R. Norum. 1982. Metabolism of very long-chain monounsaturated fatty acids (22:1) and the adaptation to their presence in the diet. *J. Lipid Res.* **23**: 243-256.
39. Hagve, T. A., and B. O. Christophersen. 1986. Evidence for peroxisomal retroconversion of adrenic acid (22:4(n-6)) and docosahexaenoic acids (22:6(n-3)) in isolated liver cells. *Biochim. Biophys. Acta.* **875**: 165-173.
40. Gronn, M., E. Christensen, T. A. Hagve, and B. O. Christophersen. 1991. Peroxisomal retroconversion of docosahexaenoic acid (22:6(n-3)) to eicosapentaenoic acid (20:5(n-3)) studied in isolated rat liver cells. *Biochim. Biophys. Acta.* **1081**: 85-91.
41. Osmundsen, H., and K. Bjornstad. 1985. Inhibitory effects of some long-chain unsaturated fatty acids on mitochondrial β -oxidation. *Biochem. J.* **230**: 329-337.
42. Willumsen, N., S. Hexeberg, J. Skorve, M. Lundquist, and R. K. Berge. 1993. Docosahexaenoic acid shows no triglyceride-lowering effects but increases the peroxisomal fatty acid oxidation in liver of rats. *J. Lipid Res.* **34**: 13-22.
43. Aarsland, A., M. Lundquist, B. Borretsen, and R. K. Berge. 1990. On the effect of peroxisomal β -oxidation and carnitine palmitoyl-transferase activity by eicosapentaenoic acid in liver and heart from rats. *Lipids.* **25**: 546-548.
44. Demoz, A., N. Willumsen, and R. K. Berge. 1992. Eicosapentaenoic acid at hypotriglyceridemic dose enhances the hepatic antioxidant defense in mice. *Lipids.* **27**: 968-971.
45. Holmer, G., C. E. Hoy, and D. Kirstein. 1982. Influence of partially hydrogenated vegetable and marine oils on lipid metabolism in rat liver and heart. *Lipids.* **17**: 585-593.
46. Conway, J. G., R. C. Cattley, J. A. Popp, and B. E. Butterworth. 1989. Possible mechanisms in hepatocarcinogenesis by the peroxisome proliferator di(2-ethylhexyl)phthalate. *Drug Metab. Rev.* **21**: 65-102.
47. Reddy, J. K., J. R. Warren, M. K. Reddy, and N. D. Lalwani. 1982. Hepatic and renal effects of peroxisome proliferators: biological implications. *Ann. NY Acad. Sci.* **386**: 81-110.
48. Kawashima, Y., A. Hirose, Y. Hirose, T. Adachi, and H. Kozuka. 1986. Inducing effects of clofibrac acid on 1-acylglycerophosphorylcholine acyltransferase in kidney and intestinal mucosa of rats. *Biochim. Biophys. Acta.* **875**: 549-553.
49. Katoh, H., Y. Kawashima, H. Watanuki, H. Kozuka, and H. Isono. 1987. Effects of clofibrac acid and tiadenol on cytosolic long-chain acyl-CoA hydrolase and peroxisomal beta-oxidation in liver and extrahepatic tissues of rats. *Biochim. Biophys. Acta.* **920**: 171-179.
50. Fringes, B., and A. Reith. 1982. Time course of peroxisome biogenesis during adaptation to mild hyperthyroidism in rat liver. *Lab. Invest.* **47**: 19-26.
51. Kerckaert, I., A. Claeys, W. Just, A. Cornelis, and F. Roels. 1989. Automated image analysis of rat liver peroxisomes after treatment with thyroid hormones: changes in number, size and catalase reaction. *Micron Microsc. Acta.* **20**: 9-18.
52. Vamecq, J., F. Roels, C. Van den Branden, and J. P. Draye. 1987. Peroxisomal proliferation in heart and liver of mice receiving chlorpromazine, ethyl 2(5(4-chlorophenyl)pentyl)oxiran-2-carboxylic acid or high fat diet: a biochemical and morphometrical comparative study. *Pediatr. Res.* **22**: 748-754.
53. Horie, S., H. Ishii, and T. Suga. 1981. Changes in peroxisomal fatty acid oxidation in the diabetic rat liver. *J. Biochem.* **90**: 1691-1696.
54. Roels, F. 1991. Peroxisomes, a Personal Account. VUB-Press, Brussels.
55. Riede, U. N., P. R. Riede, R. Horn, R. Batthiany, G. Kiefer, and W. Sandritter. 1978. Mechanisms of adaptation of hepatocytes to a chronic hypothyroidism (a cytochemical and morphometrical study). *Pathol. Res. Pract.* **162**: 398-419.
56. Leaf, A., and P. C. Weber. 1988. Cardiovascular effects of n-3 fatty acids. *N. Engl. J. Med.* **318**: 549-557.
57. Norseth, J., and M. S. Thomassen. 1983. Stimulation of microperoxisomal β -oxidation in rat heart by high-fat diets. *Biochim. Biophys. Acta.* **751**: 312-320.
58. 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.

59. Norseth, J. 1980. Increased β -oxidation of erucic acid in perfused hearts from rats fed clofibrate. *Biochim. Biophys. Acta.* 617: 183-191.
60. Lammi-Keefe, C. J., P. V. J. Hegarty, and P. B. Swan. 1981. Effect of starvation and refeeding on catalase and superoxide dismutase activities in skeletal and cardiac muscles from 12-month-old rats. *Experientia.* 37: 25-27.
61. Crescimanno, M., M. G. Armata, L. Rausa, M. C. Gueli, C. Nicotra, and N. D'Alessandro. 1989. Cardiac peroxisomal enzymes and starvation. *Free Rad. Res. Commun.* 7: 67-72.
62. Yokota, S., and K. Asayama. 1990. Peroxisomes of the rat cardiac and soleus muscles increase after starvation. A biochemical and immunocytochemical study. *Histochemistry.* 93: 287-293.
63. Yokota, S., and K. Asayama. 1992. Proliferation of myocardial peroxisomes in experimental rat diabetes: a biochemical and immunocytochemical study. *Virchows Arch. [B]* 63: 43-49.
64. Chernenko, G. A., J. A. Barrowman, K. T. Kean, G. R. Herzberg, and K. M. W. Keough. 1989. Intestinal absorption and lymphatic transport of fish oil (MaxEPA) in the rat. *Biochim. Biophys. Acta.* 1004: 95-102.
65. Grataroli, R., J. Vamecq, J. H. Poupaert, J. Léonardi, E. Termine, H. Lafont, and G. Nalbone. 1992. Effects of dietary n-6/n-3 ratios on lipid and prostaglandin E2 metabolism in rat gastric mucosa. *J. Lipid Mediators.* 5: 227-236.
66. Li, J., M. G. Wetzel, and P. J. O'Brien. 1992. Transport on n-3 fatty acids from the intestine to the retina in rats. *J. Lipid Res.* 33: 539-548.
67. Mohammed, B. S., T. A. Hagve, and H. Sprecher. 1990. The metabolism of 20- and 22-carbon unsaturated acids in rat heart and myocytes as mediated by feeding fish oil. *Lipids.* 25: 854-858.
68. Beard, M. E., and A. B. Novikoff. 1969. Distribution of peroxisomes (microbodies) in the nephron of the rat: a cytochemical study. *J. Cell Biol.* 42: 501-518.
69. Roels, F., and S. Goldfischer. 1979. Cytochemistry of human catalase. The demonstration of hepatic and renal peroxisomes by a high temperature procedure. *J. Histochem. Cytochem.* 27: 1471-1477.
70. Zaar, K. 1992. Structure and function of peroxisomes in the mammalian kidney. *Eur. J. Cell. Biol.* 59: 233-254.
71. Van den Branden, C., D. Verbeelen, and F. Roels. 1990. Peroxisomal β -oxidation in the rat kidney during increased work load. *Eur. J. Cell. Biol.* 53: 53.
72. Ohno, S. 1985. Peroxisomes of the kidney. *Int. Rev. Cytol.* 95: 131-162.
73. Kramar, R., K. Kremser, M. Hohenegger, and M. Mayer. 1986. Fatty acyl-CoA oxidase in rat kidney and liver after application of thyroxine. *Enzyme.* 35: 27-33.
74. Issemann, I., R. Prince, J. Tugwood, and S. Green. 1992. A role for fatty acids and liver fatty acid binding protein in peroxisome proliferation? *Biochem. Soc. Trans.* 20: 824-827.
75. Vamecq, J., L. Vallée, P. Lechène de la Porte, M. Fontaine, D. De Craemer, C., Van den Branden, H., Lafont, R. Grataroli, and G. Nalbone. 1993. Effect of various n-3/n-6 fatty acid ratio contents of high fat diets on rat liver and heart peroxisomal and mitochondrial β -oxidation. *Biochim. Biophys. Acta.* 1170: 151-157.