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Induction of Peroxisomal β-Oxidation in Rat Liver by High-Fat Diets

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Liver peroxisomes were prepared by using a Percoll gradient in a vertical rotor. β-Oxidation was measured in peroxisomes isolated from livers of rats fed on either high- (15% by wt.) or low- (5% by wt.) fat diets. The feeding of high-fat diets gave a 1.4–2.4-fold increase in total liver peroxisomal β-oxidation, and a similar increase in specific activity. A 1.5–4.5-fold increase was seen in the specific activity of purified peroxisomal preparations. The reasons for these increases are discussed.

Lazarow & de Duve (1976) have reported the presence of a β-oxidation system in hepatic peroxisomes. The activity of this system is markedly enhanced by clofibrate [ethyl 2-(4-chlorophenoxy)-2-methylpropionate] (Lazarow & de Duve, 1976) and di-(2-ethylhexyl)phthalate (Osumi & Hashimoto, 1978), both of which have hypolipidaemic effects. We have found that peroxisomes are particularly well suited to chain shortening of long mono-unsaturated fatty acids (Osmundsen *et al.*, 1979). The shortening of these fatty acid chains in liver is increased when rats are fed on diets containing partially hydrogenated marine oils (Thomassen *et al.*, 1978; Christiansen *et al.*, 1979). It is therefore important to determine the effects of the amount and composition of dietary fat on fatty acid oxidation in hepatic peroxisomes.

Experimental

Materials

β-NAD^+ (grade III) was obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Palmitoyl-CoA was synthesized and characterized as described previously (Neat & Osmundsen, 1979). Partially hydrogenated marine oils from capellin (*Mallotus villosus*) and soya-bean oil (*Glycine hispida*) were obtained from DeNoFa and Lilleborg Co., Fredrikstad, Norway. Percoll was purchased from Pharmacia Fine Chemicals AB, Uppsala, Sweden. Other reagents were of analytical grade or the highest purity available.

Animals and diets

Male Wistar rats (90–100g) were fed on semisynthetic diets for up to 17 days. These diets have been described elsewhere (Thomassen *et al.*, 1978), and were here modified to contain either 15% by wt. of partially hydrogenated marine oils or soya-bean oil, or 5% by wt. of soya-bean oil.

Isolation of peroxisomes

Peroxisomes have previously been isolated by vertical-rotor centrifugation by using a sucrose gradient (Neat & Osmundsen, 1979). To avoid problems associated with highly hyperosmotic sucrose gradients, we have in the present work used a Percoll gradient for peroxisomal purification. Gradients are formed, and organelles separated, during a single centrifugation step. Peroxisomal β-oxidation is readily measured in the fractionated gradient.

A subcellular fraction L of liver homogenate was prepared as described by Neat & Osmundsen (1979). A sample (40–90mg of protein) of this preparation was layered on to 29ml of a 50% (v/v) solution of Percoll containing 250mM-sucrose, 2mM-Mops (4-morpholinepropanesulphonic acid), 1mM-EGTA and 0.1% (v/v) ethanol, at pH7.2. A cushion of 60% (w/w) sucrose and an overlay of 100mM-sucrose were also used. Centrifugation was carried out at $63000\,g_{av.}$ for 30min in a Sorvall OTD-65 ultracentrifuge, in the TV-850 rotor. Ten fractions of volume 3ml were collected from the top of the gradient. The pooled peak fractions of peroxisomal activity were diluted with 2vol. of 250mM-sucrose, and centrifuged at $32000\,g_{av.}$ for 15min. The supernatant was removed by aspiration, and the sediment resuspended in the remaining medium. This fraction typically contained 1.5% of the protein found in the initial 10% homogenate. The recoveries of marker enzyme activities from the 10% homogenate were as follows: urate oxidase

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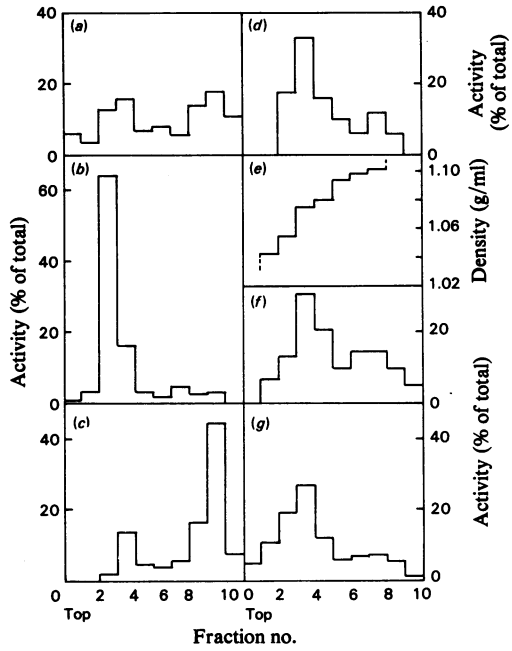

Fig. 1. *Typical profiles of marker enzymes from a self-generated Percoll gradient after centrifugation in a vertical rotor*

A subcellular fraction L from the liver of a rat fed on a diet containing partially hydrogenated marine oil for 15 days was centrifuged in the TV-850 rotor as described in the Experimental section. The gradient was fractionated into ten 3ml fractions, which were assayed. Densities are expressed in g/ml at 20°C, and enzyme activities and protein concentrations as percentages of the amount in the whole gradient. (*a*) Protein; (*b*) rotenone-insensitive cytochrome *c* redutase; (*c*) cytochrome *c* oxidase; (*d*) urate oxidase; (*e*) density; (*f*) palmitoyl-CoA-dependent NAD^+ reduction; (*g*) catalase.

(EC 1.7.3.3) 21%; catalase (EC 1.11.1.6) 7%; cytochrome *c* oxidase (EC 1.9.3.1) 2.5%; rotenone-insensitive NADPH–cytochrome *c* reductase (EC 1.6.2.4) 0.8%; *β-N*-acetyl-D-glucosaminidase (EC 3.2.1.30) 3.1%. The specific activity of urate oxidase increased 14-fold relative to that in the 10% homogenate. Fig. 1 shows the distribution of marker enzyme activities in a fractionated gradient obtained with a rat that had been fed on partially hydrogenated marine oil diet for 15 days.

Enzyme assays

Enzyme marker activities and palmitoyl-CoA-dependent NAD^+ reduction were measured as described previously (Neat & Osmundsen, 1979). The presence of marked mitochondrial contamination, as seen in a 10% liver homogenate, does not facilitate reliable and quantitative measurements of peroxisomal β-oxidation. Purified peroxisomal fractions were therefore assayed. Lazarow & de Duve (1976) have reported that β-oxidation enzymes from peroxisomes appear to be soluble and are released from damaged peroxisomes in a similar fashion to catalase. Assuming that the enzymes from animals fed on fat diets behave in a similar manner, the recovery of catalase in purified preparations can be taken to reflect the recovery of β-oxidation activity. From measurements of the two enzymes in peak peroxisomal fractions and from measurement of catalase activity in the 10% homogenate, it is therefore possible to estimate the total liver peroxisomal β-oxidation.

Proteins were measured by using the Bio-Rad protein-assay kit (Bio-Rad Laboratories). The Mann–Whitney U test was used to measure the significance of differences between mean values.

Results and Discussion

Data showing the change in total liver peroxisomal β-oxidation as a function of time on the diet are presented in Table 1. From day 5 onwards the group fed on the diet containing partially hydrogenated marine oils had a significantly higher rate of liver peroxisomal β-oxidation than either of the two other groups fed on soya-bean-oil diets ($P < 0.014$ on days 5 and 10, $P < 0.002$ on day 17). There was also, however, a significantly higher rate of β-oxidation in the group fed on the high-fat (soya-bean-oil) diet than in the groups fed on the low-fat (soya-bean-oil) diet at day 17 ($P < 0.014$). When these results are expressed per g of liver protein (see Table 1), per g of liver or per g body wt. these differences remain significant (results not shown).

In the two groups fed on high-fat diets the liver specific activities increased initially, before levelling off at a higher activity. The group fed on the low-fat diet also showed an initial increase, which after 5 days declined to a value not markedly different from the starting specific activity. The feeding of high-fat diets increased the rate of peroxisomal β-oxidation (expressed per mg of liver protein) 1.4-fold (soya-bean oil) and 2.4-fold (partially hydrogenated marine oils). This is markedly lower than the 7–8-fold increase commonly observed when rats are treated with clofibrate (Lazarow & de Duve, 1976). It is possible, however, that peroxisomal β-oxidation may be further increased by diets containing more fat than those used in this study.

Our experiments were carried out with young rapidly growing rats (90–100g body wt. on day 0, 180–200g on day 17). Blouin *et al.* (1977) reported that about 2.5% of liver protein in adult rat is peroxisomal. It is, to our knowledge, not clear whether this proportion is also present at birth and during growth. β-Oxidation in the isolated peroxi-

Table 1. *Effect of diet on liver peroxisomal β-oxidation*

Rats were fed on diets containing 15% by wt. of partially hydrogenated marine oil, 15% by wt. of soya-bean oil or 5% by wt. of soya-bean oil (low-fat). The liver peroxisomal β-oxidation activity was estimated as described in the Experimental section. Palmitoyl-CoA-dependent NAD^+ reduction was measured in the presence of 10 μM-palmitoyl-CoA, as described in the Experimental section. The tabulated values represent mean values ± S.E.M. obtained with at least four animals.

		Peroxisomal β-oxidation					
		Total activity (μmol of NAD^+ reduced/min per liver)			Specific activity (nmol of NAD^+ reduced/min per mg of liver protein)		
Time on diet (days)	Diet ...	Low fat	Soya-bean oil	Marine oil	Low fat	Soya-bean oil	Marine oil
0		1.4 ± 0.2	1.4 ± 0.2	1.4 ± 0.2	1.5 ± 0.2	1.5 ± 0.2	1.5 ± 0.2
5		1.6 ± 0.2	2.2 ± 0.3	4.1 ± 0.2	2.1 ± 0.3	2.3 ± 0.4	3.4 ± 0.5
10		1.9 ± 0.4	2.6 ± 0.6	5.9 ± 0.6	1.6 ± 0.2	2.0 ± 0.3	3.9 ± 0.4
17		2.8 ± 0.3	4.0 ± 0.6	7.0 ± 0.8	1.7 ± 0.2	2.4 ± 0.3	4.0 ± 0.4

somal fraction was therefore measured in animals fed for 14–17 days. The group fed on partially hydrogenated marine oil had a specific activity of 24.3 ± 7.5 nmol of NAD^+ reduced/min per mg of peroxisomal protein (mean value ± S.D. for six animals). The mean value obtained for four animals fed on the high-soya-bean-oil diet was 8.0 ± 1.4 nmol/min per mg, and for four animals fed on the low-fat diet the corresponding mean specific activity was 5.4 ± 1.5 nmol/min per mg. These results show that the groups fed on partially hydrogenated marine oil had a mean specific activity about 4.5 times higher than that of the group fed on the low-soya-bean-oil diet. The diet containing partially hydrogenated marine oil therefore gives a markedly higher increase in specific activity of peroxisomal β-oxidation when this is expressed per mg of peroxisomal protein (4.5-fold) than the 2.5-fold increase observed when expressed per mg of liver protein (see Table 1). This is not the case with the diet containing soya-bean oil. The reason for this is not clear, but it is possible that the diet containing partially hydrogenated marine oil may affect other organelles. Clofibrate, for example, has been shown to increase the hepatic content of mitochondria (Kurup *et al.*, 1970).

Partially hydrogenated marine oil appears to be more effective than soya-bean oil in inducing peroxisomal fatty acid oxidation. It is particularly rich in very-long-chain mono-unsaturated fatty acids (Lambertsen *et al.*, 1971), whereas soya-bean oil only contains minute amounts of these fatty acids (Houtsmuller, 1978). The possibility that the composition of dietary fat may be important requires further investigation.

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References

Blouin, A., Bolender, R. P. & Weibel, E. R. (1977) *J. Cell Biol.* **72**, 441–455

Christiansen, R. Z., Christiansen, E. N. & Bremer, J. (1979) *Biochim. Biophys. Acta* **573**, 417–429

Houtsmuller, U. M. T. (1978) *Fette, Seifen, Anstrichtm.* **80**, 162–169

Kurup, C. K. P., Aithal, H. N. & Ramasarma, T. (1970) *Biochem. J.* **116**, 773–779

Lambertsen, G., Myklestad, H. & Brækken, O. R. (1971) *J. Am. Oil. Chem. Soc.* **48**, 389–391

Lazarow, P. B. & de Duve, C. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2043–2046

Neat, C. E. & Osmundsen, H. (1979) *Biochem. J.* **180**, 445–448

Osmundsen, H., Neat, C. E. & Norum, K. R. (1979) *FEBS Lett.* **99**, 292–296

Osumi, T. & Hashimoto, T. (1978) *J. Biochem. (Tokyo)* **83**, 1361–1365

Thomassen, M. S., Strøm, E., Christiansen, E. N. & Norum, K. R. (1978) *Lipids* **14**, 58–65