Peroxisomal palmitoyl-CoA oxidation in the Zucker rat

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The effects of 3 or 6 days of starvation on hepatic peroxisomal palmitoyl-CoA oxidation were examined in adult lean and obese Zucker rats. When expressed either per mg of DNA or per total liver, obese rats had almost 2-fold higher oxidation rates than the lean rats. Within 6 days of starvation rates fell by 50% among both phenotypes. When data were expressed per 100g body wt., lean and obese rats had similar rates, falling from a mean of 0.57 to 0.28 $\mu\text{mol/min}$ per 100g body wt. within 6 days of starvation. Peroxisomal oxidative changes paralleled mitochondrial β -oxidative changes.

Differences in lipid metabolism between lean and obese Zucker rats may contribute to the development of obesity (Nosadini *et al.*, 1980; McCune *et al.*, 1981). The decreased whole-body oxygen consumption of the obese Zucker rat compared with lean littermates suggests an abnormality in oxidation (Bray, 1969). Some more recent work has specifically examined hepatic fatty acid oxidation. Triscari *et al.* (1982) and McCune *et al.* (1981), using isolated hepatocytes, and Fukuda *et al.* (1982), using perfused liver, reported that oxidation of fatty acids was less among obese than among lean Zucker rats. In addition, the ability of the hepatocytes to oxidize fatty acids and to produce oxo acids was impaired in starved obese Zucker rats. On the other hand, mitochondrial fatty acid oxidation is not different when expressed per mg of mitochondrial protein, but is actually increased in obese Zucker rats when expressed as total hepatic capacity (Brady & Hopfel, 1983). These data may be reconciled with the hepatocyte data when the number of cells per g of liver is considered.

Long-chain fatty acids can be oxidized by peroxisomes (Lazarow & de Duve, 1976; Lazarow, 1978; Osmundsen *et al.*, 1979). *In vitro*, oxidation of palmitoyl-CoA is maximal when the ratio of acyl-CoA to free CoA is large (Osmundsen & Neat, 1979). This has led to the suggestion that hepatic peroxisomal oxidation of palmitate represents an overflow system for the mitochondria. Murphy *et al.* (1979) have reported that hepatic peroxisomal fatty acid oxidation is increased in genetically obese mice in the fed state, but the effects of starvation were not examined. A limited body of data exists which

suggests that starvation can influence peroxisomal β -oxidation (Ishii *et al.*, 1980; Thomassen *et al.*, 1982). The present study examines the effect of starvation on hepatic peroxisomal β -oxidation in lean and obese Zucker rats.

Materials and methods

Adult lean and obese littermate pairs of Zucker rats were purchased from Fab Laboratories, New City, NY, U.S.A. The rats were individually housed in stainless-steel wire-bottom cages with access to food and water *ad libitum*. The diet was a lactalbumin-based semi-purified diet (Brady & Hopfel, 1983). In brief, the diet composition was (g/kg): lactalbumin, 210.0; L-arginine hydrochloride, 1.3; corn starch, 149.7; sucrose, 492.0; corn oil, 50.0; cellulose, 50.0; choline bitartrate, 2.0; ethoxyquin, 0.01; vitamin mixture, 10.0; and mineral mixture, 35.0. The composition of the mineral and vitamin mixtures were those recommended by the American Institute of Nutrition (1977, 1980) for the growing rat. Rats were maintained in our animal facility for a minimum of 1 week before use. The rats were either fed (five pairs), or starved for 3 (four pairs) or 6 days (seven pairs). Rats were killed by decapitation and the livers rapidly removed. Total liver was weighed and a 1–2g sample homogenized for isolation of peroxisomes (Osmundsen & Neat, 1979). Final centrifugation was at 79 000 g_{av} , for 30 min (approx. $1850 \times 10^7 \text{ rad}^2 \cdot \text{s}^{-1}$) in a vertical rotor (type VTi 50; Beckman Instruments, Palo Alto, CA, U.S.A.) with a 50% Percoll (Pharmacia Fine Chemicals, New York, NY, U.S.A.) self-generating gradient containing 250mM-sucrose, 20mM-Mops (4-morpholine-propanesulphonic acid), 5mM-EGTA and 0.1%

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ethanol, pH 7.2. The gradient (total volume 30 ml) was fractionated into ten 3 ml fractions.

The peroxisomal peak was determined by using catalase as a marker enzyme (Baudhuin *et al.*, 1964). Catalase activity was also determined in the initial 10% (w/v) homogenate. Catalase units are expressed in terms of the first-order rate constant. The position of the peroxisomal peak was confirmed in selected samples by using urate oxidase activity (Leighton *et al.*, 1968). Glutamate dehydrogenase (Leighton *et al.*, 1968) and citrate synthase (Srere, 1969) activities were used as markers to assess the mitochondrial contamination of the peroxisomal peak. The peak peroxisomal fraction was assayed for palmitoyl-CoA-dependent NAD⁺ reduction as described by Mannaerts *et al.* (1979), by using a dual-wavelength spectrophotometer (Aminco DW 2a; Travenol Laboratories, Chicago, IL, U.S.A.) at 340 nm with a 400 nm reference wavelength. Units were expressed as μmol of NAD⁺ reduced/min. Protein and DNA concentrations had been determined in the homogenate (Brady & Hoppel, 1983). Protein was also determined directly in the peroxisomal peak fraction (Bradford, 1976).

The data were analysed as a 2×3 factorial design with unequal replication by the method of Federer &

Zelen (1966) adapted to our laboratory computer system. The correlation between peroxisomal and mitochondrial (Brady & Hoppel, 1983) palmitate oxidation was assessed by linear regression (Steele & Torrie, 1960).

Results and discussion

Some 40–50% of catalase activity within the gradient was confined to a single 3 ml fraction which corresponded to the peak of urate oxidase activity. Less than 3% of glutamate dehydrogenase and citrate synthase activities contaminated this fraction.

Data generated in the present study are presented in Table 1. Liver weight was roughly 2-fold higher among obese rats and, although this difference was maintained with starvation, a nearly 50% decrease in liver weight resulted by day 6 of starvation. Expressed as g per 100 g body wt., liver was heavier initially among the obese rats, but values converged with those observed for the lean rats by day 7 of starvation.

Ishii *et al.* (1980) reported a decreased hepatic catalase activity per mg of protein with starvation in

Table 1. *Effects of starvation on liver weight, catalase activity and peroxisomal palmitoyl-CoA-dependent NAD⁺ reduction rates among lean and obese Zucker rats*

Data are presented as means $\pm$ S.E.M. for the numbers of rats indicated. Data were analysed as a 2×3 factorial arrangement of treatments with unequal replication. Statistically significant effects ($P < 0.05$) are designated as 'P' for phenotype and 'S' for starvation. Catalase units are expressed in terms of the first-order rate constant. Peroxisomal protein was determined from catalase recoveries. Units for palmitoyl-CoA-dependent NAD⁺ reduction are μmol of NAD⁺ reduced/min.

	Period of starvation (days) ... 0		3		6		Significant effects
	Lean	Obese	Lean	Obese	Lean	Obese	
No. of rats	5	5	4	4	7	7	—
Liver wt.							
(g)	8.4 $\pm$ 1.1	21.0 $\pm$ 2.2	4.8 $\pm$ 0.2	15.0 $\pm$ 0.5	5.1 $\pm$ 0.5	10.2 $\pm$ 0.8	P,S
(g/100 g body wt.)	2.9 $\pm$ 0.1	4.4 $\pm$ 0.3	2.4 $\pm$ 0.1	3.2 $\pm$ 0.4	2.4 $\pm$ 0.1	2.3 $\pm$ 0.2	P,S
Catalase activity							
(units/mg of peroxisomal protein)	6.7 $\pm$ 1.3	7.2 $\pm$ 1.9	12.7 $\pm$ 3.7	9.2 $\pm$ 2.4	15.4 $\pm$ 1.9	15.0 $\pm$ 1.9	S
(units/g of liver)	114 $\pm$ 17	91 $\pm$ 8	65 $\pm$ 11	65 $\pm$ 7	103 $\pm$ 18	105 $\pm$ 21	—
(units/mg of DNA)	87 $\pm$ 23	163 $\pm$ 40	35 $\pm$ 5	110 $\pm$ 24	36 $\pm$ 7	59 $\pm$ 9	P,S
(units/liver)	839 $\pm$ 163	2007 $\pm$ 294	303 $\pm$ 56	920 $\pm$ 198	541 $\pm$ 121	1050 $\pm$ 206	P,S
Peroxisomal protein							
(mg/g of liver)	14 $\pm$ 3	12 $\pm$ 4	4 $\pm$ 1	6 $\pm$ 1	5 $\pm$ 1	5 $\pm$ 2	S
(mg/liver)	93 $\pm$ 17	287 $\pm$ 124	16 $\pm$ 1	88 $\pm$ 18	27 $\pm$ 10	50 $\pm$ 17	P,S
Peroxisomal palmitoyl-CoA-dependent NAD ⁺ reduction							
(munits/mg of peroxisomal protein)	9.4 $\pm$ 2.6	6.4 $\pm$ 1.6	13.1 $\pm$ 4.0	15.2 $\pm$ 5.0	18.7 $\pm$ 5.3	14.0 $\pm$ 3.3	—
(munits/g of liver)	191 $\pm$ 54	114 $\pm$ 46	51 $\pm$ 19	105 $\pm$ 33	105 $\pm$ 21	113 $\pm$ 41	—
(munits/mg of DNA)	152 $\pm$ 57	228 $\pm$ 82	24 $\pm$ 5	202 $\pm$ 87	32 $\pm$ 6	66 $\pm$ 22	P,S
(units/liver)	1.4 $\pm$ 0.4	2.6 $\pm$ 0.9	0.2 $\pm$ 0.1	1.4 $\pm$ 0.4	0.5 $\pm$ 0.1	1.2 $\pm$ 0.5	P,S
(units/100 g body wt.)	0.53 $\pm$ 0.15	0.61 $\pm$ 0.14	0.12 $\pm$ 0.04	0.30 $\pm$ 0.11	0.28 $\pm$ 0.04	0.29 $\pm$ 0.09	S

Wistar rats. We observed increased catalase activity per mg of protein as a function of starvation, although it did decline when expressed per total liver. Activity per mg of DNA also followed this pattern. Considerable change in liver weight occurred with starvation. However, DNA content, which reflects cell number, remained constant per total liver (Brady & Hoppel, 1983). Hence, changes in catalase activity per total liver paralleled activity per mg of DNA. Thus activity per total liver or per mg of DNA, though not numerically the same, reflects activity per cell, and, since cell number did not change, per total cells. Further, obese Zucker rats had higher liver weights than did lean rats. This would produce both a higher total content of peroxisomal protein per liver and a higher content per cell among the obese rats. Regardless of the genotype, there was a substantial decline in the peroxisomal protein content per cell or per liver as a function of starvation.

The patterns of peroxisomal palmitoyl-CoA-dependent NAD⁺ reduction were quite similar to those observed for catalase. Obesity did not affect activity per mg of protein or per g of liver. Activity per liver, calculated from catalase recovery (Lazarow & de Duve, 1976), was a minimum of 2-fold higher among obese rats, and this difference was maintained when expressed per mg of DNA. Although the obese rats had elevated liver weights per 100 g body wt. through 3 days of starvation, there was no effect of obesity on palmitoyl-CoA oxidation rates expressed per 100 g body wt. That is, although differences in palmitate oxidation were observed, as a result of obesity, per rat, these differences are lost when the weight of the rat was considered.

Starvation resulted in a slight, but not statistically significant, increase in palmitate oxidation per mg of peroxisomal protein. Activity per g of liver was also unaffected by starvation. Activity per liver decreased sharply between 0 and 3 days of starvation, with no additional change between 3 and 6 days. Activity per mg of DNA also decreased with starvation, lean rats exhibiting the greatest change between 0 and 3 days, whereas obese rats showed a more delayed response, reaching a minimum by 6 days. Murphy *et al.* (1979) have found that hepatic palmitate-oxidation rates are higher for obese mice than for lean littermates when expressed per total liver. Unlike the Zucker rat, the obese (*ob/ob*) mice also showed elevated activity per mg of protein and per g of liver.

Starvation resulted in a 50% decrease in oxidative capacity per total liver, per mg of DNA and per 100 g body wt. within 3 days of starvation. Mannaerts *et al.* (1979) have reported peroxisomal palmitoyl-CoA oxidation per g of liver among Wistar rats to increase within 24 h of starvation,

with no change in total hepatic capacity. Ishii *et al.* (1980) and Thomassen *et al.* (1982) have both reported increases in oxidation with starvation in Wistar rats previously fed on commercial lab chow diets. However, Thomassen *et al.* (1982) found no change in oxidation if the animals were previously fed on high-fat (25%, by wt.) diets. The lactalbumin diet given in the present studies contained only 5% fat (as corn oil), suggesting that factors other than the fat content of the diet may be involved in starvation-related changes. Each of the previous studies used immature male Wistar rats. The present work used adult Zucker rats. Thus, although the strain of rat may be critical, it is also possible that the age or sexual maturity of the rats may play a role in their response to starvation.

Peroxisomal palmitate oxidation rates paralleled mitochondrial palmitoylcarnitine-oxidation rates, which are reported elsewhere (Brady & Hoppel, 1983). We have suggested that changes in the mitochondrial capacity per liver reflect loss of hepatic mitochondrial protein mass with starvation. The same statement is also true with regard to the peroxisome. That is, DNA content, which reflects cell number, remained constant. Changes in peroxisomal palmitate oxidation per mg of DNA reflected loss of peroxisomal protein per cell, and this is precisely what was indicated by the catalase-based estimates of peroxisomal protein.

A significant linear correlation was observed between mitochondrial and peroxisomal oxidation rates per liver ($r = 0.68$; 30 degrees of freedom). The metabolic relationship for peroxisomes and mitochondria is unknown. The peroxisome has a specificity for long-chain acyl-CoA species. Further, it is not capable of complete oxidation to acetate (Lazarow & de Duve, 1976). Indeed, no more than five acetate molecules are formed per molecule of palmitate. With palmitoyl-CoA as substrate, peroxisomal β -oxidation proceeds maximally when the ratio of free CoA to palmitoyl-CoA is small (Osmundsen & Neat, 1979). As mentioned above, this would argue for the potential of the peroxisomal oxidation to be maximal when mitochondrial oxidation is saturated. Such a situation may exist in the obese Zucker rat, when fatty livers are observed. Although peroxisomal palmitate-oxidation rates per mg of protein are not different for lean and obese rats, there is an increase in the peroxisomal protein per mg of DNA. It is noteworthy that both mitochondrial and peroxisomal palmitate oxidation decrease concomitantly with starvation. These data would at least suggest that both organelles are simultaneously controlled. Whether this is accomplished by the same metabolic message remains to be determined.

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References

- American Institute of Nutrition (1977) *J. Nutr.* **107**, 1340–1348
- American Institute of Nutrition (1980) *J. Nutr.* **110**, 1726
- Baudhuin, P., Beaufay, H., Rahman-Li, Y., Sellinger, O. Z., Wattiaux, R., Jacques, P. & de Duve, C. (1964) *Biochem. J.* **92**, 179–184
- Bradford, M. (1976) *Anal. Biochem.* **72**, 248–253
- Brady, L. J. & Hoppel, C. L. (1983) *Am. J. Physiol.* in the press
- Bray, G. A. (1969) *Experientia* **25**, 1100–1101
- Federer, W. T. & Zelen, M. (1966) *Biometrics* **22**, 525–552
- Fukuda, N., Azain, M. & Ontko, J. (1982) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **41**, 1216
- Ishii, H., Horie, S. & Suga, T. (1980) *J. Biochem. (Tokyo)* **87**, 1855–1858
- Lazarow, P. B. (1978) *J. Biol. Chem.* **253**, 1522–1528
- Lazarow, P. B. & de Duve, C. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2043–2046
- Leighton, F., Poole, B., Beaufay, H., Baudhuin, P., Coffey, J. W., Fowler, S. & de Duve, C. (1968) *J. Cell Biol.* **37**, 482–493
- Mannaerts, G. P., Debeer, L. J., Thomas, J. & DeSchepper, P. J. (1979) *J. Biol. Chem.* **254**, 4585–4595
- McCune, S., Durant, P., Jenkins, P. & Harris, R. (1981) *Metab. Clin. Exp.* **30**, 1170–1178
- Murphy, P. A., Krahling, J. A., Gee, R., Kirke, J. R. & Tolbert, N. E. (1979) *Arch. Biochem. Biophys.* **193**, 179–185
- Nosadini, R., Ursini, F., Tessari, P., Garotti, M., DeBiasi, F. & Tiengo, A. (1980) *Eur. J. Clin. Invest.* **10**, 113–118
- Osmundsen, H. & Neat, C. E. (1979) *FEBS Lett.* **107**, 81–85
- Osmundsen, H., Neat, C. E. & Norum, K. R. (1979) *FEBS Lett.* **99**, 292–296
- Srere, P. (1969) *Methods Enzymol.* **13**, 3–11
- Steele, R. G. D. & Torrie, J. H. (1960) *Principles and Procedures of Statistics*, pp. 99–106, McGraw-Hill Book Co., New York
- Thomassen, M. W., Christiansen, E. N. & Norum, K. R. (1982) *Biochem. J.* **206**, 195–202
- Triscari, J., Greenwood, M. R. C. & Sullivan, A. C. (1982) *Metab. Clin. Exp.* **31**, 223–228