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Study of some factors controlling fatty acid oxidation in liver mitochondria of obese Zucker rats

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Livers of genetically obese Zucker rats showed, compared with lean controls, hypertrophy and enrichment in triacylglycerols, indicating that fatty acid metabolism was directed towards lipogenesis and esterification rather than towards fatty acid oxidation. Mitochondrial activities of cytochrome *c* oxidase and monoamine oxidase were significantly lower when expressed per g wet wt. of liver, whereas peroxisomal activities of urate oxidase and palmitoyl-CoA-dependent NAD^+ reduction were unchanged. Liver mitochondria were able to oxidize oleic acid at the same rate in both obese and lean rats. For reactions occurring inside the mitochondria, e.g. octanoate oxidation and palmitoyl-CoA dehydrogenase, no difference was found between both phenotypes. Total carnitine palmitoyl-, octanoyl- and acetyl-transferase activities were slightly higher in mitochondria from obese rats, whereas the carnitine content of both liver tissue and mitochondria was significantly lower in obese rats compared with their lean littermates. The carnitine palmitoyltransferase I activity was slightly higher in liver mitochondria from obese rats, but this enzyme was more sensitive to malonyl-CoA inhibition in obese than in lean rats. The above results strongly suggest that the impaired fatty acid oxidation observed in the whole liver of obese rats is due to the diminished transport of fatty acids across the mitochondrial inner membrane via the carnitine palmitoyltransferase I. This effect could be reinforced by the decreased mitochondrial content per g wet wt. of liver. The depressed fatty acid oxidation may explain in part the lipid infiltration of liver observed in obese Zucker rats.

INTRODUCTION

In the liver of genetically obese Zucker rats, lipogenesis and fatty acid esterification are enhanced (Martin, 1974; Taketomi *et al.*, 1975; Bray, 1977; Sullivan *et al.*, 1977; Godbole & York, 1978), whereas fatty acid oxidation is depressed (Nosadini *et al.*, 1980; Fukuda *et al.*, 1982; McCune *et al.*, 1981; Triscari *et al.*, 1982; Azain & Martin, 1983). From studies on isolated hepatocytes, Triscari *et al.* (1982) suggested that mitochondrial β-oxidation could be impaired in obese rats. However, using liver mitochondria, Brady & Hoppel (1983*a*) failed to demonstrate any difference in O_2 consumption required for fatty acid oxidation, and proposed that the depressed oxidation in isolated hepatocytes from obese rats could be due to a decreased mitochondrial content. On the other hand, we have previously reported that carnitine palmitoyltransferase I was much more sensitive to malonyl-CoA inhibition in mitochondria from obese than from lean Zucker rats (Clouet *et al.*, 1985). It can therefore be proposed that the decreased fatty acid oxidation by hepatocytes from obese rats could merely be due to the presence of high malonyl-CoA concentration rather than to an actual decrease in the oxidative capacity of mitochondria. This agrees with the conclusions of Brady & Hoppel (1983*a*), but in their studies the oxidative capacity of mitochondria was estimated indirectly through the O_2 consumption.

The aim of the present work was to re-evaluate the oxidative capacity of liver mitochondria from obese Zucker rats in comparison with the lean littermates by measuring the activity of several enzymes involved directly in fatty acid oxidation. Since the oxidation of fatty acids also occurs in peroxisomes (Lazarow & de Duve, 1976), we have attempted to prepare a mitochondrial fraction poor in peroxisomes. On the other hand, we have determined the fatty acid oxidation by peroxisomes in liver homogenates of both phenotypes in order to estimate the possible contribution of these organelles to the impaired fatty acid oxidation of the intact liver from obese rats.

EXPERIMENTAL

Animals

Obese (*fa/fa*) and lean (*Fa/-*) male Zucker rats were bred in the Centre de Sélection et d'Elevage d'Animaux de Laboratoire, C.N.R.S., Orléans-la-Source, France. They were 11 weeks old at receipt and were given a standard laboratory chow (AO3; UAR, 91360 Epinay-sur-Orge, France) *ad libitum* for 1 week. After food deprivation for 16 h, they were stunned and decapitated at 08:00 h.

Liver lipids

The livers of two groups of obese and lean rats were perfused *in situ* with 20 ml of ice-cold 0.9% NaCl through the portal vein; they were then removed, blotted with paper, weighed and cut into small pieces. A 2 g sample was extracted for lipid determination (Folch *et al.*, 1957). A known amount of tripentadecanoin was added as internal standard to the total lipids, and the methyl esters of fatty acids were analysed by g.l.c. (Bézard & Ouedraogo, 1980) for total fatty acids of liver

Abbreviation used: CAT, carnitine acyltransferase.

Table 1. Mitochondrial and peroxisomal activities, nuclear content in liver homogenates and mitochondrial fractions from obese and lean Zucker rats

Values are means ± S.E.M. ($n = 5$). Both enzymatic activities were measured at 25 °C and expressed as μmol/min for cytochrome *c* oxidase and nmol/min for catalase. Total activity or content of homogenate refers to activity or content per g of liver; total activity or content of mitochondria was calculated from the total mitochondrial fraction actually obtained; specific activity refers to activity per mg of protein of homogenate or mitochondria. Statistical significance (Student's *t* test) of the difference between obese and lean rats is indicated by: (NS) $P > 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Phenotype...	Obese	Lean
Cytochrome *c* oxidase		
Total activity in:		
Homogenate	51.7±6.2*	68.6±3.6
Mitochondria	3.2±0.4*	4.9±0.3
Specific activity in:		
Homogenate	0.247±0.025*	0.315±0.015
Mitochondria	0.687±0.028 (NS)	0.681±0.018
Catalase		
Total activity in:		
Homogenate	40.6±4.5*	53.9±2.7
Mitochondria	0.121±0.015 (NS)	0.092±0.011
Specific activity in:		
Homogenate	0.200±0.020*	0.247±0.015
Mitochondria	0.023±0.009 (NS)	0.013±0.005
DNA (μg)		
Total content in:		
Homogenate	5101±148***	6891±82
Mitochondria	38±5**	55±4
Content per mg of protein of:		
Homogenate	24.3±1.2**	31.5±1.6
Mitochondria	9.1±0.3 (NS)	8.2±0.1

and mitochondria. The triacylglycerol fraction containing the internal standard was separated by t.l.c., and the fatty acids were analysed by g.l.c. for total triacylglycerol fatty acids of liver.

Mitochondrial fraction

Two other groups of obese and lean rats were killed as above. The liver of each animal was removed and immediately cooled to 4 °C in 0.25 M-sucrose/10 mM-triethanolamine/1 mM-EDTA, pH 7.4. It was then chopped, rinsed several times, blotted, weighed, and homogenized in 15 vol. of the same mixture with two strokes of a Teflon pestle in a Potter–Elvehjem homogenizer. In order to prepare a highly purified mitochondrial fraction poor in peroxisomes, mitochondria were isolated from the postnuclear supernatant as follows. The homogenate was centrifuged for 10 min at 3 °C and 500 *g* ($r_{av.}$ 7 cm) and the supernatant was discarded. The pellet (P1) was diluted with the sucrose medium to the same volume as the initial homogenate, resuspended with two strokes of the pestle in the homogenizer, re-centrifuged as above, and the pellet (P2) was retained. The same operation was repeated for the pellets P2 and P3; the last pellet (P4), which essentially contained nuclei, was discarded, and the supernatant, which contained mitochondria, was centrifuged at 1300 *g* for 10 min. The pellet thus obtained was resuspended in the same medium without EDTA and re-centrifuged at 1300 *g* for 10 min. The last pellet contained the mitochondrial fraction, which represented only 5–8% of the initial hepatic cytochrome *c* oxidase activity (Table 1), but the short-time homogenization procedures and the low-speed centrifugations maintained good mitochondrial membrane integrity. The procedure effectively discarded peroxisomes, as estimated by catalase activity, and left in the mitochondrial fraction a very small amount of nuclei, as indicated by DNA measurements (Labarca & Paigen, 1980), which did not modify the specific activity of cytochrome *c* oxidase in the preparations of both phenotypes (Table 1). For the estimation of CAT-I activity, mitochondria were isolated by the same procedure as above, but the homogenization mixture was replaced throughout the operation by 0.25 M-mannitol/5 mM-Hepes buffer (pH 7.4)/1 mM-EGTA, as indicated by Bremer (1981).

Enzyme assays

The liver mitochondrial content was estimated in homogenates by the activities of monoamine oxidase (EC 1.4.3.4) for the external membrane (Weissbach *et al.*, 1960) and of cytochrome *c* oxidase (EC 1.9.3.1) for the internal membrane (Beaufay *et al.*, 1974). The activity of total CAT (CAT-I and CAT-II) (EC 2.3.1.21) was measured in the presence of 40 μM-oleoyl- or -palmitoyl-CoA (Bieber *et al.*, 1972), except that Hepes buffer was used instead of Tris (Seccombe *et al.*, 1978) and 4,4′-dithiobispyridine instead of 5,5′-dithionitrobenzoic acid (Ramsay & Tubbs, 1975). The activity of the outer carnitine palmitoyltransferase I was measured at 25 °C as described by Bremer (1981), in the presence of [*methyl*-^{3}H]carnitine, with slight modifications (Clouet *et al.*, 1985); the palmitoyl[^{3}H]carnitine was extracted with butan-1-ol (Bremer & Norum, 1967) and then counted for radioactivity in Picofluor 30 (Packard Instrument Co.) in a Packard 300 C scintillation counter. The mitochondrial activity of acyl-CoA dehydrogenase was

measured as described by Korsrud *et al.* (1976) in a medium containing in addition 35 μM-palmitoyl-CoA, 2 mM-KCN, 15 μM-rotenone, 10 μM-antimycin to block the respiratory chain, and 0.05% (v/v) Triton X-100 to obtain maximal enzymic activity.

Peroxisomal activities of liver were measured in homogenates by using catalase (EC 1.11.1.6) as described by Aebi (1974) and urate oxidase (EC 1.7.3.3) as described by Leighton *et al.* (1968); the fatty acyl-CoA oxidase activity, which is the first oxidative reaction in peroxisomes, was detected by the palmitoyl-CoA-dependent H_2O_2 generation coupled to the peroxidation of methanol in an antimycin A-insensitive reaction, as described by Inestrosa *et al.* (1979), and formaldehyde was measured by the procedure of Nash (1953); the peroxisomal fatty acid-oxidizing system reported by Lazarow & de Duve (1976) was determined by CN^--insensitive palmitoyl-CoA-dependent NAD^+ reduction, as described by Bronfman *et al.* (1979), in the presence of 75 μM-palmitoyl-CoA.

Measurements of total carnitine content of homogenates and mitochondria were performed as indicated by Ramsay & Tubbs (1975).

Protein determination

The protein content of homogenate and organelle preparations was determined by the biuret method (Layne, 1957). However, because the high fat content in some livers disturbed the protein measurements, all the samples, as well as the calibration solutions, were preliminarily saponified for 1 h at 70 °C with 2 M-NaOH to give accurate protein determinations.

Fatty acid oxidation

The incubation medium for mitochondria consisted of 20 mM-potassium phosphate, pH 7.4, 50 mM-KCl, 4 mM-$MgCl_2$, 1 mM-ATP, 50 μM-CoA, 0.2 mM-L-malate, and 50 μM-potassium [1-^{14}C]oleate bound to bovine albumin (fraction V; fatty acid-free) in a molar ratio ranging from 1.5 to 9; L-carnitine (1 mM) was added to the medium when indicated.

When oleate was replaced by 200 μM-potassium [1-^{14}C]octanoate, only phosphate, KCl and ATP were used and in concentrations as above; the same amount of 1.2% (w/v) albumin was added to each incubation medium.

The reaction was initiated with 1 mg of mitochondrial protein in 2 ml of medium maintained at 35 °C with gentle shaking. After 10 min for oleate or 5 min for octanoate, the reaction was stopped by adding 0.1 ml of 10 M-HCl and then 8 ml of 10% (w/v) $HClO_4$ in order to precipitate proteins and long-chain fatty acids which were still intact or bound to CoA or carnitine. The medium was then filtered on a Millipore filter (pore size 0.45 μm). In assays with octanoic acid, which is soluble in dilute $HClO_4$, 2 mg of unlabelled octanoic acid was added and the fatty acid was extracted from the filtrate by washing three times with diethyl ether. The radioactivity of the filtrates, determined in Picofluor 15 (Packard), corresponds to labelled ketone bodies, acetate molecules bound to CoA or carnitine and to intermediary products of the tricarboxylic acid cycle. These molecules, which are derived from fatty acid oxidation, were called 'acid-soluble products' (ASP). CO_2 was trapped in Hyamine (Packard). This production was negligible for oleate oxidation.

Materials

The chemicals were from Prolabo (Paris, France) and Merck (Darmstadt, Germany). The biochemicals were purchased from Sigma (St. Louis, MO, U.S.A.), L- and D-carnitine were given by Dr. C. Cavazza of Sigma-Tau (Pomezia, Italy). [1-^{14}C]Oleic acid ($C_{18:1}$) was from CEA (Saclay, France), and [1-^{14}C]octanoic acid ($C_{8:0}$) was from NEN France SARL (Paris, France); labelled fatty acids were diluted with the corresponding unlabelled fatty acids to a specific radioactivity of 2 Ci/mol, and used as potassium salts. Oleoyl- and palmitoyl-CoA were prepared by the method of Goldman & Vagelos (1961).

Statistics

The significance of phenotypic differences was assessed by Student's *t* test.

RESULTS

The liver weights of obese rats were significantly higher, even when expressed per 100 g body wt. (Table 2). The protein content per g wet wt. of tissue was lower and the lipid concentration was 15 times higher, mainly because of the higher triacylglycerol content. The activities of monoamine oxidase and of cytochrome *c* oxidase (Table 3) were respectively 40 and 25% lower in obese rats as compared with the lean controls. The activities of acyl-CoA oxidase and catalase were also

Table 2. Body and liver weights, protein and lipid contents of liver from obese and lean Zucker rats

Results are means ± S.E.M. Rats ($n = 6$ in each group) were 12 weeks old. Protein and lipids were measured as described in the Experimental section. Statistical significance (Student's *t* test) of the difference between both phenotypes is indicated by ***$P < 0.001$.

Rats	Body wt. (g)	Liver wt. (g)	Liver wt. (g/100 g body wt.)	Liver protein (mg/g wet wt. of tissue)	Liver lipids (mg of total fatty acids/g wet wt. of tissue)	Liver lipids (mg of triacylglycerol fatty acids/g wet wt. of tissue)
Obese	423 ± 10***	16.3 ± 0.4***	3.9 ± 0.1***	238 ± 9***	67.0 ± 2.9***	35.2 ± 3.0***
Lean	275 ± 6	8.1 ± 0.3	2.90 ± 0.06	280 ± 5	29.9 ± 1.1	2.3 ± 0.3

Table 3. Enzymic activities in liver homogenates of obese and lean Zucker rats

Values are means ± S.E.M. ($n = 5$). Enzyme activities are expressed per g of liver as follows: μmol of substrate transformed/min at 37 °C for monoamine oxidase, urate oxidase, fatty acid oxidase and CN^--insensitive palmitoyl-CoA-dependent NAD^+ reduction (peroxisomal fatty acid-oxidizing system); μmol/min at 25 °C for cytochrome *c* oxidase; and nmol/min at 25 °C for catalase. Statistical significance (Student's *t* test) of the difference between obese and lean animals is indicated by: (NS) $P > 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

	Mitochondrial activities		Peroxisomal activities			
Phenotype	Monoamine oxidase	Cytochrome *c* oxidase	Urate oxidase	Catalase	Fatty acid oxidase	Peroxisomal fatty acid-oxidizing system
Obese	0.63 ± 0.02***	63.5 ± 3.0**	3.36 ± 0.15(NS)	38.9 ± 3.0**	0.75 ± 0.08**	1.06 ± 0.06 (NS)
Lean	1.02 ± 0.03	82.2 ± 3.1	3.67 ± 0.15	54.3 ± 2.1	1.17 ± 0.07	1.21 ± 0.05

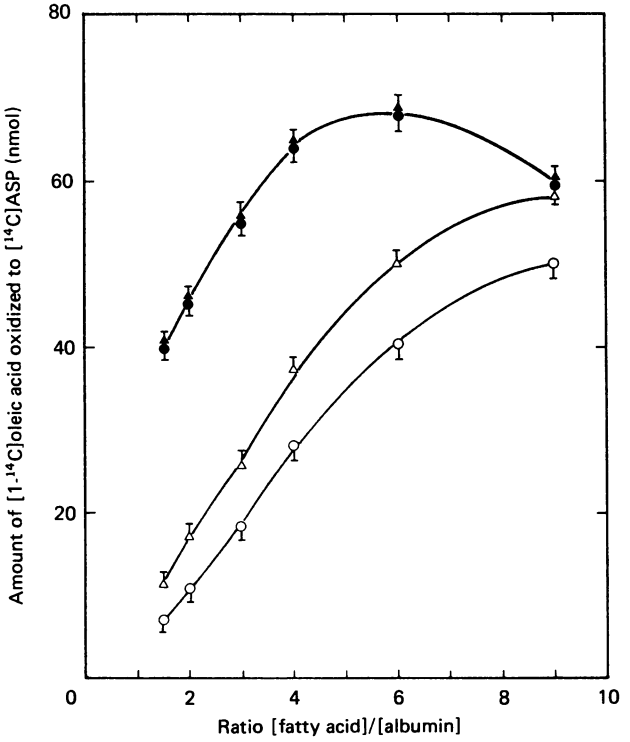

Fig. 1. Oleate oxidation by liver mitochondria from obese and lean Zucker rats

The incubation conditions are described in the Experimental section. Potassium oleate (100 nmol) and 1 mg of mitochondrial protein were used in 2 ml of medium. Albumin was added in several molar ratios of [fatty acid]/[albumin] ranging from 1.5 to 9. Results are expressed as nmol of ^{14}C-labelled fatty acid whose radioactivity was recovered as acid-soluble products (ASP) at the end of incubation. ●, ○, Obese rats; ▲, △, lean rats; ●, ▲, in the presence of exogenous carnitine; ○, △ in its absence. Results are means ± S.E.M. ($n = 5$ for each group). Each point represents the mean of five duplicated determinations; each determination corresponds to one mitochondrial preparation from one rat. Bars show S.E.M.

decreased in the liver of obese rats (Table 3). However, no significant differences were found in the activities of urate oxidase and CN^--insensitive palmitoyl-CoA-dependent NAD^+ reduction (Table 3).

The oxidation of oleic acid by mitochondrial fractions represents the total sequence from the activation by CoA at the external membrane side to the reactions occurring in the matrix. Fig. 1 illustrates the results of [1-^{14}C]oleate oxidation by liver mitochondria from obese and lean rats in presence of decreasing amounts of albumin, i.e. increasing ratio (*R*) of fatty acid/albumin. In the absence of exogenous carnitine, oleate oxidation increased with the ratio values up to $R = 9$, but was less in the obese rats whatever the *R* values. Oleate oxidation was strongly stimulated by the addition of carnitine, particularly for the low fatty acid/albumin ratio, and was similar in both phenotypes for all the *R* values. The activities of the total carnitine acyltransferase (CAT-I + CAT-II) and of CAT-I alone were slightly higher in mitochondria of obese rats (Table 4). Similar results were obtained for carnitine octanoyl- and acetyl-transferase activities. The oxidation of octanoic acid, which mostly crosses the mitochondrial inner membrane by a non-enzymic mechanism, was studied to provide information about the steps located inside the mitochondria. Table 4 shows that [1-^{14}C]octanoate oxidation was very similar in mitochondria from obese and lean rats, and this similarity was still reflected in the activities of the first acyl-CoA dehydrogenase from the β-oxidative sequence, observed in both groups.

On the other hand, mitochondria from obese rats contained 40% less total carnitine per mg of protein. When carnitine concentrations were referred to the two marker enzyme activities, they were similarly 30% lower. In the liver, the carnitine concentration was 40 and 30% lower in obese rats when expressed per g wet wt. of tissue and per g of protein respectively. Lastly, the lipid concentration of mitochondrial preparations per mg of protein was 40% higher in obese than in lean rats (Table 4).

DISCUSSION

In the liver exogenous or endogenous fatty acids are oxidized or esterified in triacylglycerols, which are normally secreted as very-low-density lipoproteins. Since the hepatic production of very-low-density lipoproteins is higher in obese than in lean rats (Schonfeld & Pfleger, 1971), the accumulation of lipids in liver of obese rats results from an increased fatty acid synthesis (Martin, 1974; Godbole & York, 1978), eventually reinforced by a depressed fatty acid oxidation. A study of the activity

Table 4. Enzymic activities, carnitine and lipid contents of liver mitochondria from obese and lean Zucker rats

Values are means ± S.E.M. ($n = 6$). Lipid and carnitine contents were measured as specified in the Experimental section. All activities of carnitine-dependent enzymes were assessed at 25 °C and were expressed as nmol/min per mg of mitochondrial protein. Octanoate oxidation was performed for 5 min at 35 °C in the presence of 1 mg of mitochondrial protein, and results are expressed as nmol of [^{14}C]octanoic acid, the radioactivity of which was recovered in the CO_2 and in the ether-extracted acid-soluble products. Acyl-CoA dehydrogenase activity was assessed at 30 °C with palmitoyl-CoA and was expressed as nmol of 2,6-dichlorophenolindophenol reduced/min per mg of mitochondrial protein. Statistical significance (Student's t test) of the difference between obese and lean rats is indicated by: (NS) $P > 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Phenotype...	Obese	Lean
Total carnitine acyltransferase (CAT I+II)		
With oleoyl-CoA	8.20±0.33 (NS)	7.50±0.25
With palmitoyl-CoA	12.86±0.84**	9.40±0.24
Carnitine palmitoyltransferase I	2.87±0.10*	2.55±0.06
Carnitine octanoyltransferase	29.3±2.1**	17.9±1.2
Carnitine acetyltransferase	1.45±0.11 (NS)	1.83±0.39
Octanoate oxidation:		
100 μM	9.7±0.5 (NS)	8.6±0.4
200 μM	12.1±0.6 (NS)	10.5±0.5
Acyl-CoA dehydrogenase with palmitoyl-CoA:		
10 μM	9.3±0.2 (NS)	8.4±0.3
35 μM	11.5±0.7 (NS)	10.4±0.4
Carnitine content in mitochondria (nmol/g of protein)	87±6***	138±4
Carnitine content in liver (nmol/g wet wt. of tissue)	192±7***	267±5
Lipid content in mitochondria (mg of total fatty acids/g of protein)	158±8***	114±1

of organelles involved in fatty acid oxidation can thus provide information about the cause of lipid accumulation.

Among the peroxisomal enzymes directly involved in fatty acid oxidation (Lazarow & de Duve, 1976), acyl-CoA oxidase activity was slightly lower per g of liver in obese than in lean rats, whereas palmitoyl-CoA-dependent NAD^+ reduction, which reflects more truly the overall activity of the peroxisomal β-oxidative pathway (Inestrosa *et al.*, 1979), appeared to be similar in both phenotypes; thus the peroxisomal oxidative system does not seem to be implicated in the decrease in the hepatic capacity for fatty acid oxidation in obese Zucker rats, which strengthens the conclusions of Brady & Hoppel (1983*b*).

The activity of the mitochondrial enzymes per g of liver, which reflects the amount of mitochondria, was significantly lower in obese than in lean rats, but the lower mitochondrial mass per g of tissue is compensated by the larger liver weight. Nevertheless, the increased mitochondrial activity for fatty acid oxidation, when expressed per total liver, does not result in an enhanced fatty acid oxidation in perfused liver experiments (Nosadini *et al.*, 1980; Fukuda *et al.*, 1982).

The capacities of liver mitochondria for oleate oxidation were very similar in the presence of exogenous carnitine in both phenotypes, whether albumin was added in amounts considered as physiological (low fatty acid/albumin ratios) or when fatty acids act as mitochondrial membrane detergents (high ratios). These results can be explained by the similarity of the main intermediary steps of fatty acid oxidation studied in both phenotypes, i.e. the carnitine-dependent reactions and the intramitochondrial activities, octanoate oxidation and acyl-CoA dehydrogenase activity (Table 4). These data compare with those reported by Brady & Hoppel (1983*a*), who used different biochemical methods, but do not agree with those of Triscari *et al.* (1982), who reported a lower oxidation of octanoate and palmitoylcarnitine in hepatocytes of obese rats, and suggesting that β-oxidation could be impaired in liver of this phenotype. Since in the hepatocyte these substrates can follow various metabolic pathways, fatty acid synthesis after previous β-oxidation to octanoate (McGarry & Foster, 1971) or hydrolysis to give palmitoylcarnitine (Berg & Farstad, 1979), the lower oxidation of these substrates in isolated hepatocytes of obese rats could be due to a lower rate of transfer of fatty acids through the mitochondrial inner membrane. This transfer is regulated by CAT-I activity (McGarry *et al.*, 1977) and it is strongly inhibited by malonyl-CoA (McGarry & Foster, 1980; Saggerson & Carpenter, 1981; Robinson & Zammit, 1982). Malonyl-CoA concentration was higher in both liver and hepatocytes of obese rats than of lean rats (Azain *et al.*, 1985; Malewiak *et al.*, 1985). Moreover, CAT-I is much more sensitive to malonyl-CoA inhibition in the liver of obese than of lean rats (Clouet *et al.*, 1985). Both peculiarities markedly decrease the entry of fatty acids inside the mitochondria of obese rats and may be responsible for the decrease in carnitine concentration in liver and mitochondria (Table 4) as a consequence of the lower need for carnitine.

Oleate oxidation did not occur in the absence of exogenous carnitine when fatty acids are bound to sufficient amounts of albumin (Fig. 1), but, when albumin was in lower amounts, the mitochondrial inner membrane became more permeable because of unbound fatty acids, which also avoid the CAT-I step and were more easily oxidized. This carnitine-independent oxidation, which was lower in the obese than in the lean rat,

could be explained by the higher lipid content of the mitochondrial preparations from obese rats (Table 4), either by diluting the detergent effect of unbound fatty acids, or because of changes in composition of membrane phospholipids of mitochondria from obese rats. The composition of the inner-membrane phospholipids should be investigated in connection with the sensitivity of CAT-I to malonyl-CoA inhibition.

In summary, as the oxidative activity of liver mitochondria isolated from their cellular environment is the same in both obese and lean rats, liver malonyl-CoA concentrations which increase in all lipogenesis states and the enhanced sensitivity of CAT-I to malonyl-CoA appear to be essential for explaining the defect in fatty acid oxidation observed in liver of obese rats in spite of the hepatic hypertrophy and the high mitochondrial content. In this regard, oxidative impairment seems to be due to an enhanced lipogenesis rather than to decreased mitochondrial capacity.

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