Biochem. J. (1988) 249, 801–806 (Printed in Great Britain)

Fatty acid metabolism in hepatocytes isolated from rats adapted to high-fat diets containing long- or medium-chain triacylglycerols

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Fatty acid oxidation and synthesis were studied in isolated hepatocytes from adult rats adapted for 44 days on low-fat, high-carbohydrate (LF), diet or high-fat diets, composed of long-chain (LCT) or medium-chain (MCT) triacylglycerols. The rates of [1-^{14}C]octanoate oxidation were almost similar in each group studied, whereas the oxidation of [1-^{14}C]oleate was 50% lower in the LF group than in animals adapted to high-fat diets. The rates of oleate oxidation are inversely correlated with the rates of lipogenesis. However, it seems unlikely that [malonyl-CoA] itself represents the sole mechanism involved in the regulation of oleate oxidation during long-term LCT or MCT feeding, since: (1) despite a 3-fold higher concentration of malonyl-CoA in MCT-fed rats than in LCT-fed ones, the rates of oleate oxidation are similar; (2) when malonyl-CoA concentration is increased after stimulation of lipogenesis (by adding lactate+pyruvate) in MCT-fed rats, to a level comparable with that of the LF group, the rate of oleate oxidation remains 55% higher than that measured under similar conditions in the LF-fed rats; (3) in the LF group, the 90% decrease in malonyl-CoA concentration [by 5-(tetradecyloxy)-2-furoic acid] is not associated with a stimulation of oleate oxidation. By contrast, the sensitivity of carnitine palmitoyltransferase I (CPT I) to malonyl-CoA is markedly decreased in the LCT- and MCT-fed rats, by 90% and 70% respectively. The relevance of this decrease in the sensitivity of CPT I is discussed.

INTRODUCTION

Long-term feeding with MCT diets has received considerable attention in recent years, mainly for the treatment of obesity or disorders in lipid absorption (for reviews, see Sickinger, 1975; Bach & Babayan, 1982). Although the metabolic adaptation to MCT diets is largely documented in the literature (for review, see Bach & Babayan, 1982), some aspects of the hepatic fatty acid metabolism remain controversial, especially with respect to lipogenesis. Indeed, it has been reported that, during MCT feeding, the rate of lipogenesis in liver slices and lipogenic enzyme activities were either slightly decreased (Wiley & Leveille, 1973; Yeh & Zee, 1976; Lavau & Hashim, 1978; Crozier *et al.*, 1987) or increased 2-fold (Takase *et al.*, 1977; Takase & Hosoya, 1986). In fact, the rate of lipogenesis is of particular importance in the regulation of hepatic fatty acid oxidation, since malonyl-CoA, the product of the reaction catalysed by acetyl-CoA carboxylase, is a potent inhibitor of CPT I, the rate-limiting enzyme of LCFA oxidation (for review, see McGarry & Foster, 1980; Girard & Malewiak, 1986). It has been reported that the 50% decrease in liver lipogenesis, secondary to acute feeding with MCT, was associated with a 2-fold increase in hepatic palmitate oxidation in rat liver slices (Yeh & Zee, 1976), which is in agreement with the concept of an inverse relationship between lipogenesis and LCFA oxidation (McGarry & Foster, 1980). However, the viability of the liver-slice technique could be questioned, since, in similar conditions, a 90% inhibition of liver lipogenesis after acute LCT feeding did not enhance hepatic palmitate oxidation (Yeh & Zee, 1976).

The aim of the present work was to study the rates of fatty acid oxidation and lipogenesis in hepatocytes isolated from MCT-adapted rats, in comparison with those obtained during long-term feeding of LF or LCT diets.

EXPERIMENTAL

Animals and experimental design

Male Wistar rats (Janvier, Le Genest, France) weighing 150–180 g were used. They were housed in individual cages (22±2 °C; light from 07:00 to 19:00 h) and adapted to a LF diet (% of metabolizable energy: 21.3 protein; 67.9 carbohydrate; 10.8 fat) for 21 days before the beginning of the study. Then they were divided in three groups according to the diets. Each group was fed *ad libitum* for 44 days as follows: a control group fed on LF diet (for composition see above); two groups fed on high-fat diets composed of either LCT (% metabolizable energy: 23.7 protein; 4.0 carbohydrate; 72.3 fat) or MCT (% of metabolizable energy: 24.9 protein; 4.2 carbohydrate; 70.9 fat). Since the fatty acid composition of triacylglycerols (see below) shows that more than 82% of fatty acids are unsaturated in the LCT diet, whereas more than 95% of fatty acids are saturated in the MCT diet, an additional group of animals was fed on a high-fat diet in which unsaturated LCT is replaced by hydrogenated soya-bean oil (saturated LCFA: 98%). Food intake and body weight were recorded every 2 or 4

Abbreviations used: LCT, long-chain triacylglycerols; MCT, medium-chain triacylglycerols; LCFA, long-chain fatty acid; LF, low-fat, high-carbohydrate; CPT, carnitine palmitoyltransferase; TOFA, 5-(tetradecyloxy)-2-furoic acid.

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days respectively. Energy intakes of the rats fed on high-fat diets were similar (305 ± 6 kJ/day), but 13% lower than that for the LF diet. Nevertheless, the weight gain of rats fed on the MCT diet was 28% less than that of the groups fed on saturated or unsaturated LCFA and 39% less than that of the LF group. For more details concerning the composition of the body-weight gain, see Crozier *et al.* (1987).

Isolation and incubation of hepatocytes

After 44 days of experimental feeding, hepatocytes were isolated at 09:00 h, i.e. in the post-absorptive period. Hepatocytes were prepared by the method of Berry & Friend (1969), as modified by Krebs *et al.* (1974). For each experimental group, 20 mM-glucose was included in the perfusion medium, and the cell density was adjusted to 5×10^6 hepatocytes/ml, except for malonyl-CoA determinations, where the cell density was increased to 20×10^6–25×10^6 hepatocytes/ml. Incubations of 1 ml portions of these hepatocyte suspensions were performed in duplicate at 37 °C in a final volume of 2 ml of Krebs–Henseleit buffer (pH 7.4). The viability of the isolated cells was high (87 ± 2%) and did not fluctuate according to the diet.

Fatty acid oxidation, esterification and ketogenesis

These were measured in the presence of 1 mM-carnitine, with either [1-^{14}C]octanoate (1 mM; 0.1 μCi/μmol) or [1-^{14}C]oleate (0.3 mM; 0.5 μCi/μmol) as precursors. Both fatty acids were bound to 2% (w/v) defatted albumin. In order to compare fatty acid oxidation and lipogenesis under similar conditions, labelled fatty acids were added to the incubation medium after a 30 min preincubation period (see below) and then further incubated for 30 min. For studies of fatty acid oxidation and ketogenesis, the incubation was ended by adding 0.25 ml of $HClO_4$ (40%, v/v). The productions of $^{14}CO_2$ and labelled acid-soluble products were determined as described by Mannaerts *et al.* (1979) for [^{14}C]oleate and by McGarry & Foster (1971) for [^{14}C]octanoate. For studies of fatty acid esterification, incubations were ended by centrifugation for 30 s at 3000 *g*. The lipids from the chloroform/methanol cell-pellet extracts were separated by t.l.c. as described by Duée *et al.* (1985).

Lipogenesis

Hepatocytes were preincubated for 30 min in the absence or in the presence of lactate (10 mM) plus pyruvate (1 mM) with or without TOFA (0.1 mM). Then 250 μCi of ^3H_2O and oleate (0.3 mM) were added, and hepatocytes were further incubated for 30 min. The incubations were ended by centrifugation for 30 s at 3000 *g*. Labelled fatty acids were extracted from the cell pellet as described by Stansbie *et al.* (1976).

Fatty acid oxidation (acid-soluble products and CO_2), ketone-body production subtracted from endogenous production (without exogenous fatty acid), fatty acid esterification and lipogenesis were calculated between 30 and 60 min of incubation and expressed per 10^6 hepatocytes. Neither the dry weight nor the protein content of isolated hepatocytes was affected by the diets (results not shown).

Malonyl-CoA concentration

Hepatocytes were incubated for 30 min in the presence of oleate (0.3 mM) and carnitine (1 mM), in the absence or in the presence of lactate (10 mM) plus pyruvate (1 mM), with or without TOFA (0.1 mM). The incubations were ended by adding 0.2 ml of $HClO_4$ (30%, v/v). Malonyl-CoA was assayed as described by McGarry *et al.* (1978*c*). Rat liver fatty acid synthetase was prepared as described by Stoops *et al.* (1979).

Isolation of liver mitochondria and measurements of respiration

Mitochondria were prepared as described by Mersmann *et al.* (1972). The livers were rapidly sampled and rinsed in a medium containing 220 mM-mannitol, 70 mM-sucrose, 2 mM-Hepes and 0.1 mM-EDTA, pH 7.4. All processing steps were conducted at 4 °C. After mincing, sliced livers were homogenized by two up/down strokes of a loose-fitting motor-driven Teflon pestle (Heidolph R.G.I., West Germany) at a constant speed of 50–60 rev./min. After centrifugation (successively at 700 *g* and 10000 *g*, each for 10 min), the final pellet was resuspended in the isolation buffer at a concentration of approx. 50 mg of protein/ml and kept at 4 °C. Protein was determined by the method of Lowry *et al.* (1951).

The mitochondrial respiration was measured at 30 °C, with an oxygraph (Gilson model 5/6 H) equipped with a 2 ml water-jacketed chamber and with a Clark oxygen electrode. The rates of O_2 consumption from glutamate (5 mM)/malate (5 mM) in the absence (State 4) or in the presence of 90 μM-ADP (State 3) were determined as described previously (Escriva *et al.*, 1986).

Assay of CPT I

This was performed as described by Bremer (1981). The incubation medium contained 75 mM-KCl, 50 mM-mannitol, 25 mM-Hepes (pH 7.3), 2 mM-KCN, 0.2 mM-EGTA, 1 mM-dithiothreitol and 1% fat-free albumin. The total volume was 0.5 ml and the incubation temperature 30 °C. For determination of IC_{50} (concn. giving 50% inhibition), mitochondria (0.2 mg of protein/ml) were preincubated for 3 min in the presence of 80 μM-palmitoyl-CoA and in the absence or in the presence of various concentrations of malonyl-CoA (0.05–150 μM), and then further incubated for 6 min after addition of 1 mM-L-[*methyl*-^{3}H]carnitine (2 μCi/μmol). The palmitoyl[^{3}H]carnitine formed was measured after butan-1-ol extraction (Bremer, 1981). The assays were performed within 30 min after isolation of mitochondria, and were linear with time and protein concentration (results not shown). In all experiments, the formation of palmitoylcarnitine was completely suppressed by the highest malonyl-CoA concentration (150 μM), suggesting that only CPT I activity was measured, without any significant contribution of CPT II (McGarry *et al.*, 1983).

Analytical methods

Acetoacetate, 3-hydroxybutyrate, lactate and pyruvate were determined enzymically as described by Bergmeyer (1974). Glucose was assayed as described by Werner *et al.* (1970).

Chemicals

All enzymes, substrates and cofactors were obtained from Boehringer Corp. (Meylan, France). Fatty acid-free albumin, oleate, octanoate and L-carnitine were purchased from Sigma (St. Louis, MO, U.S.A.). [1-^{14}C]-Oleate, [1-^{14}C]octanoate, ^3H_2O, L-[*methyl*-^{3}H]carnitine

Table 1. Rates of oleate or octanoate oxidation in isolated hepatocytes from adult rats adapted to LF, LCT or MCT diets

The hepatocytes were isolated in the post-absorptive period from rats adapted to low-fat (LF) medium-chain triacylglycerol (MCT) or long-chain triacylglycerol (LCT) diets. After a 30 min preincubation period, [1-^{14}C]oleate plus carnitine (1 mM) or [1-^{14}C]octanoate was added. Both fatty acids were bound to fat-free albumin (final concn. 2%). Hepatocytes were further incubated for 30 min, and the rates of ketone-body production, subtracted from endogenous production, were calculated over this 30 min incubation period. The term 'total metabolized' represents the sum of 1-^{14}C-labelled fatty acid converted into acid-soluble products, CO_2 or triacylglycerol. The hydroxybutyrate/acetoacetate (HOB/AcAc) ratio refers to the respective productions over the 30 min incubation period. Other values are in nmol/30 min per 10^6 hepatocytes. Values are means ± S.E.M. for the numbers of experiments shown in parentheses; $*P < 0.01$ compared with the LF group.

Substrate added	Diet	Ketone-body production	Total 1-^{14}C-labelled fatty acid metabolized	1-^{14}C-labelled fatty acid converted into: CO_2	Total acid-soluble products	Triacyl-glycerol	HOB/AcAc
Octanoate (1 mM)	LF (7)	206±19	126±10	15±1	99±9	12±1	2.0±0.1
	MCT (11)	319±31*	141±13	10±1*	126±13	5±1	3.2±0.7
	LCT (7)	272±20*	132±11	8±1*	123±10	1±0.5	5.1±1.0*
Oleate (0.3 mM)	LF (7)	32±5	27±1	4±0.5	8±1	15±1	1.4±0.2
	MCT (11)	144±17*	29±3	3±0.5	19±2*	7±1*	1.5±0.3
	LCT (7)	166±21*	26±2	2±0.3*	21±1*	3±1*	2.4±0.4

and [^{3}H]acetyl-CoA were obtained from The Radiochemical Centre (Amersham, Bucks., U.K.). TOFA was a gift from Dr. J. Hudak (Merrell Dow Pharmaceutical, Cincinnati, OH, U.S.A.). The fatty acid compositions of the high-fat diets were as follows: LCT, $C_{16:0}$ 11.3%, $C_{18:0}$ 2.9%, $C_{18:1}$, 61.7%, $C_{18:2}$ 20.5%, $C_{20:0}$ 0.4%, $C_{20:1}$ 0.3%; MCT, $C_{8:0}$ 62.3%, $C_{10:0}$ 35.3%. They were kindly supplied by Dr. F. Mendy (Sopharga, Puteaux, France). The fatty acid composition of hydrogenated soya-bean oil was: $C_{16:0}$ 10.7%; $C_{18:0}$ 87.3%; $C_{18:1}$ 1.5%; $C_{18:2}$ 0.2%; $C_{20:0}+C_{20:1}$ 0.1%. It was kindly supplied by Dr. P. Doye (SIO, Nanterre, France).

Statistics

Results are expressed as means ± S.E.M. Statistical analyses were performed by Student's unpaired *t* test.

RESULTS AND DISCUSSION

As shown in Table 1, the rates of [1-^{14}C]octanoate metabolism are similar whatever the diets consumed, as previously reported for adult rat liver slices, after acute feeding with MCT or LCT (Yeh & Zee, 1976). However, it is noteworthy that the rates of ketogenesis from octanoate are significantly higher after long-term feeding of high-fat diets than after feeding the LF diet (Table 1). This results from a slight increase in the overall octanoate oxidation in MCT and LCT groups [respectively 136±13 ($n = 11$) and 131±10 ($n = 7$) nmol/30 min per 10^6 cells] when compared with the LF group [114±10 nmol/30 min per 10^6 cells ($n = 7$)], but also from a modification in the channelling of acetyl-CoA formed, towards ketone-body synthesis rather than the tricarboxylic acid cycle. In keeping with this, we found that $^{14}CO_2$ production amounted to 13% of the total octanoate oxidation in the LF group, but only to 6–7% in the LCT and MCT groups. This difference in the metabolic fate of acetyl-CoA formed could be linked to the decrease in liver oxaloacetate concentration after long-term feeding with LCT or MCT diets (Bach *et al.*, 1976; Crozier *et al.*, 1987). This probably results from an enhanced gluconeogenesis, as suggested either directly by Eisenstein *et al.* (1974) or by the rise in phosphoenolpyruvate carboxykinase activity (Crozier *et al.*, 1987). In keeping with this, we found that the rates of glucose production from 10 mM-lactate + 1 mM-pyruvate were 2-fold higher in the LCT and MCT groups [respectively 359±36 ($n = 7$) and 333±43 ($n = 11$) nmol/30 min per 10^6 cells] than in the LF group [147±20 nmol/30 min per 10^6 cells ($n = 7$)].

However, the partition of acetyl-CoA between ketogenesis and the tricarboxylic acid cycle is probably not the main regulatory site of ketone-body production. Indeed, when compared with ketogenesis from octanoate, the rates of ketone-body production from oleate are decreased 2-fold in the LCT and MCT groups, but markedly depressed in the LF group. Moreover, [1-^{14}C]-oleate oxidation is 50% lower in the LF group than in animals adapted to high-fat diets (Table 1). As the metabolism of octanoate seems to be independent of CPT I (McGarry & Foster, 1971), and as the carnitine ester oxidation, which is only dependent on CPT II, is as high in liver mitochondria from LF-fed rats as in those of high-fat-fed rats (Neat *et al.*, 1981; Brady & Hoppel, 1983), these results suggest that, during long-term feeding of a high-fat diet, the principal regulatory step of fatty acid oxidation and ketogenesis would be located at the level of CPT I. It has been shown in adult rat liver that the activity of CPT I was mainly controlled by two mechanisms (for reviews, see McGarry & Foster, 1980; Girard & Malewiak, 1986): (1) a decrease in the concentration of malonyl-CoA, the first committed intermediate in the lipogenic pathway; (2) a fall in CPT I sensitivity to the inhibition by malonyl-CoA.

These two possibilities will be discussed successively.

Relationship between lipogenesis and fatty acid oxidation

As previously reported by many investigators (for a review, see Romsos & Leveille, 1974), the present study

Table 2. Effects of stimulation or inhibition of lipogenesis on oleate oxidation in isolated hepatocytes from adult rats adapted to LF, MCT or LCT diets

Hepatocytes from LF-, MCT- and LCT- (see the legend of Table 1) adapted rats were preincubated for 30 min in presence of lactate (10 mM) plus pyruvate (1 mM) with or without TOFA (0.1 mM). Then [1-^{14}C]oleate (0.3 mM) + carnitine (1 mM), or ^3H_2O (250 μCi/flask) + oleate (0.3 mM) and carnitine (1 mM) were added, and hepatocytes were further incubated for 30 min. The rates of oleate oxidation and lipogenesis were calculated over this 30 min incubation period. Malonyl-CoA concentrations were determined in hepatocytes after 30 min of incubation in presence of oleate (0.3 mM) and carnitine (1 mM), with or without lactate, pyruvate or TOFA. Values are means ± S.E.M. for 6–11 determinations per group; $*P < 0.05$ and $**P < 0.01$ compared with the LF group.

Additions	Diet	Lipogenesis (nmol of ^3H_2O incorporated into fatty acids/30 min per 10^6 hepatocytes)	Malonyl-CoA concn. (nmol/10^6 hepatocytes)	[1-^{14}C]Oleate converted into total acid-soluble products + CO_2 (nmol/30 min per 10^6 hepatocytes)
None	LF	31 ± 8	0.118 ± 0.029	12 ± 1
	MCT	17 ± 3	0.085 ± 0.022	22 ± 2**
	LCT	3 ± 1*	0.033 ± 0.009*	23 ± 1**
Lactate, pyruvate	LF	53 ± 17	0.141 ± 0.018	9 ± 1
	MCT	55 ± 6	0.131 ± 0.024	13 ± 1*
	LCT	7 ± 2**	0.081 ± 0.003**	20 ± 2**
Lactate, pyruvate, TOFA	LF	2 ± 0.5	0.013 ± 0.003	13 ± 2
	MCT	3.5 ± 1.8	0.028 ± 0.008	16 ± 2
	LCT	2 ± 0.5	0.014 ± 0.004	17 ± 2*

confirms that LCT diets markedly depress the rate of hepatic lipogenesis (Table 2). By contrast, MCT feeding is much less effective in decreasing hepatic lipogenesis (Table 2), as previously reported in terms of enzyme activities (Lavau & Hashim, 1978; Crozier *et al.*, 1987). In fact, it seems unlikely that the relative decrease in lipogenesis in the MCT group (compared with the LF group; Table 2) is the result of this marginal decrease in lipogenic enzyme activities, but more likely is the consequence of the 6-fold decrease in lipogenic substrate availability. Indeed, the endogenous rate of lactate + pyruvate production was 6-fold lower in hepatocytes from MCT-fed rats than in LF-fed ones [61 ± 19 (n = 11) and 348 ± 33 (n = 7) nmol/30 min per 10^6 cells respectively], reflecting probably the lower hepatic glycogen content in the high-fat-fed rats (Crozier *et al.*, 1987). In keeping with this, the addition of lactate + pyruvate to hepatocytes isolated from MCT-fed rats restores a rate of lipogenesis similar to that found in LF-adapted rats, whereas it did not in the LCT group (Table 2).

The data in Table 2 show that under certain conditions there is an inverse relationship between lipogenesis and oleate oxidation. For instance, when lipogenesis is high (LF group), or stimulated by adding lactate + pyruvate (LF and MCT groups), the corresponding rates of oleate oxidation are low or decreased. Inversely, a low lipogenic activity is associated with a high rate of fatty acid oxidation (LCT group). Since malonyl-CoA concentrations fluctuate in parallel with the rates of lipogenesis (Table 2), as previously reported (Guynn *et al.*, 1972; McGarry *et al.*, 1978*b*), these results could suggest, at first sight, that malonyl-CoA could be involved in the regulation of oleate oxidation during long-term MCT feeding. However, it seems unlikely that this regulation implies the sole variation in hepatic malonyl-CoA concentration. Indeed, three pieces of experimental evidence support this assumption.

Firstly, despite a malonyl-CoA concentration 3-fold higher in MCT-fed rats than in LCT-fed rats (Table 2), the rates of oleate oxidation are similar. Secondly, when malonyl-CoA concentration is increased (by adding lactate + pyruvate) in hepatocytes isolated from the MCT-fed rats, to a value comparable with that of the LF group (Table 2), the rate of oleate oxidation remains 55% higher than that measured under similar condition in the LF-fed rats (Table 2). Thirdly, when the malonyl-CoA concentration is profoundly depressed by 90% in the LF-fed rats (Table 2), by adding TOFA, an inhibitor of acetyl-CoA carboxylase (Panek *et al.*, 1977; McCune & Harris, 1979), the rate of oleate oxidation is not stimulated (Table 2). It is noteworthy that this does not result from an inhibitory effect of TOFA on oleate oxidation, as reported by Otto *et al.* (1985), since this compound failed to affect significantly the oleate oxidation in MCT- or LCT-adapted rats (Table 2). Moreover, higher concentration of TOFA (0.2 mM) produced a marked stimulation of fatty acid oxidation in isolated hepatocytes from meal-fed rats (McGarry & Foster, 1979).

As the LCT diet contains a predominant proportion of unsaturated fatty acids, whereas the MCT diet does not, which in turn could explain the metabolic differences between both groups (Triscari *et al.*, 1978), a similar experiment was performed with hepatocytes isolated from rats fed on hydrogenated soya-bean oil as the sole source of fat in the diet. As in the LCT group, the rate of ketone-body production from octanoate (1 mM) in this group is twice that from oleate (0.3 mM): respectively 301 ± 20 and 128 ± 12 nmol/30 min per 10^6 cells (n = 4). Moreover, oleate oxidation is as high as in the LCT

Table 3. Respiratory control ratio (RCR) and CPT I activity and sensitivity to malonyl-CoA in liver mitochondria from LF-, MCT- and LCT-adapted rats

The RCR represents the ratio of mitochondrial O_2 consumption from glutamate (5 mM) plus malate (5 mM) in the presence (State 3) to that in the absence (State 4) of ADP (90 μM). CPT I activity was measured in the presence of 80 μM-palmitoyl-CoA and 1 mM-carnitine. IC_{50} refers to the concentration of malonyl-CoA required for 50% inhibition of CPT I activity. Values are means ± S.E.M. for seven determinations; $*P < 0.01$ compared with the LF group.

Liver source	RCR	CPT I (nmol/min per mg of protein)	IC_{50} for malonyl-CoA (μM)
LF	5.40±0.36	1.76±0.10	0.22±0.01
MCT	6.03±0.30	1.84±0.14	0.67±0.08*
LCT	5.87±0.30	2.04±0.08	1.87±0.32*

group [20±3 ($n = 4$) and 23±1 nmol/30 min per 10^6 cells; Table 2], concomitantly with a low rate of lipogenesis (6±1 nmol of ^3H_2O incorporated into fatty acids/30 min per 10^6 cells) or a low malonyl-CoA concentration (0.049±0.005 nmol/10^6 cells; $n = 5$). Even in the presence of lactate plus pyruvate, this lipogenic capacity remains low (13±2 nmol of ^3H_2O incorporated/30 min per 10^6 cells), as does the malonyl-CoA concentration (0.071±0.02 nmol/10^6 cells).

These results emphasize that the simultaneous occurrence of a high rate of oleate oxidation and an active lipogenesis is a metabolic feature of the liver of MCT-adapted rats. The present data rule out a direct effect from the saturation of fatty acids, and suggest that mechanism(s) other than changes in malonyl-CoA concentration must be involved.

CPT activity and sensitivity to malonyl-CoA inhibition

Before investigating the CPT I sensitivity to malonyl-CoA inhibition, it was important to test the intactness of the isolated liver mitochondria, since the damage of mitochondrial membrane could lead to a loss of the malonyl-CoA sensitivity (McGarry *et al.*, 1978*a*). This was performed by measuring the mitochondrial O_2 consumption from glutamate+malate in the absence (State 4), or in the presence (State 3) of ADP, which is commonly used as an indicator of mitochondrial intactness (Chance & Williams, 1955). The high-fat diets did not affect the States 3 and 4 of mitochondrial respiration (results not shown), thus leading to similar respiratory-control ratios in each group studied (Table 3) and compatible with the criteria of mitochondrial intactness. Similar data have been reported in isolated mitochondria from rat fed on high-fat diets (McMurchie *et al.*, 1983*b*; Brady & Hoppel, 1983).

As shown in Table 3, the CPT I activity (although not significant) tends to increase in the LCT-fed rats, whereas it is unaffected during long-term MCT feeding. This result is in agreement with the 50% increase in oxidation of palmitate (Veerkamp & Zevenbergen, 1986) or palmitoyl-CoA (Neat *et al.*, 1981) in isolated mitochondria from rats fed on high-fat diets composed of long-chain fatty acids. In addition to the moderate rise in CPT I activity, the LCT-fed rats exhibited a 10-fold increase in the IC_{50} for malonyl-CoA (Table 3), suggesting a large decrease in the sensitivity of CPT I to inhibition by malonyl-CoA. The IC_{50} for malonyl-CoA is also increased 3-fold in the MCT-fed rats (Table 3) and 5-fold in rats fed on hydrogenated soya-bean oil (results not shown). These results do not seem to be due to the experimental conditions used, since the values of IC_{50} for malonyl-CoA in the LF-fed rats agree well with those previously reported in similar conditions (for review, see Girard & Malewiak, 1986). This decreased sensitivity of CPT I to the inhibition by malonyl-CoA could explain why the rate of oleate oxidation is 1.5-fold higher in the MCT-fed rats than in LF-fed ones, despite similar malonyl-CoA concentrations (Table 2). In adult rat liver, it has been shown in many physiological or pathological situations that such a change in CPT I sensitivity to malonyl-CoA inhibition was dependent on the nutritional or endocrine state of the animal (for review, see Girard & Malewiak, 1986). Whether the fall in sensitivity of CPT I to malonyl-CoA during long-term fat feeding is under hormonal control and/or is the result of the diet remains to be elucidated.

However, these two aspects could be subject to discussion. At least, in terms of pancreatic hormones, which have been shown to play a crucial role in the regulation of CPT I activity and sensitivity (Harano *et al.*, 1982; Cook *et al.*, 1984; Gamble & Cook, 1985), it is noteworthy that, during the first 4 days of the consumption of the LCT or MCT diets, the insulin/glucagon molar ratio was markedly decreased when compared with the LF-fed rats (Crozier *et al.*, 1987). By contrast, at the end of the experiment, i.e. when CPT I activity and sensitivity were measured, the plasma concentration of pancreatic hormones is similar whatever the diet consumed (Crozier *et al.*, 1987). Does this early hormonal signal induce permanent modifications of the kinetic properties of CPT I, or the synthesis of an enzyme with different sensitivity to malonyl-CoA? In keeping with this, it has been shown in rat liver that administration of cycloheximide blocked the increases in CPT I activity and IC_{50} on starvation, suggesting that starvation and/or associated hormonal changes may induce the synthesis of a new enzyme (Saggerson *et al.*, 1984).

On the other hand, it has been shown in adult rat liver that re-feeding with high-carbohydrate diet totally reversed the decrease in CPT I sensitivity to malonyl-CoA induced by starvation, whereas re-feeding with high-fat diet did not (Saggerson *et al.*, 1984), suggesting that a part of the change in CPT I sensitivity could be ascribable to the high fat content of the diet. Indeed, it was reported that high-fat feeding may induce marked changes in the lipid composition of the mitochondrial inner membrane, especially in increasing the membrane fluidity (McMurchie *et al.*, 1983*a*). In keeping with this, it has been shown that, during starvation or diabetes, the liver mitochondrial membrane fluidity was decreased and that the associated decrease in CPT I sensitivity to malonyl-CoA could be the result of the inability of malonyl-CoA to increase the rigidity of the membrane as it did in the fed rats (Brady *et al.*, 1985, 1986).

Although elucidation of both of these hypotheses needs further experiments, the present work emphasizes that long-term feeding with LCT or MCT diet provides a good model in which to study the regulation of the complex relationship between CPT I and malonyl-CoA.

We are indebted to Dr. M. Lavau, who kindly performed the purification of rat liver fatty acid synthetase, to E. Toussaint, who takes care of the animals and diets, to C. Kohl, for her technical assistance, to P. Robin, who performed the polarographic measurements of mitochondrial respiration, and to M. Fernandez, for her careful preparation of the manuscript.

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Received 28 July 1987/21 September 1987; accepted 5 October 1987