Response to starvation of hepatic carnitine palmitoyltransferase activity and its regulation by malonyl-CoA

Sex differences and effects of pregnancy

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1. Hepatic carnitine palmitoyltransferase activity was measured over a range of concentrations of palmitoyl-CoA and in the presence of several concentrations of the inhibitor malonyl-CoA. These measurements were made in mitochondria obtained from the livers of fed and starved (24 h) virgin female and fed and starved pregnant rats. 2. In the fed state overt carnitine palmitoyltransferase activity was significantly lower in virgin females than in age-matched male rats. 3. Starvation increased overt carnitine palmitoyltransferase activity in both virgin and pregnant females. This increase was larger than in the male and was greater in pregnant than in virgin females. 4. In the fed state pregnancy had no effect on the Hill coefficient or the $[S]_{0.5}$ when palmitoyl-CoA was varied as substrate. Pregnancy did not alter the sensitivity of the enzyme to inhibition by malonyl-CoA. 5. Starvation decreased the sensitivity of the enzyme to malonyl-CoA. The change in sensitivity was similar in male, virgin female and pregnant rats. 6. The possible relevance of these findings to known sex differences and changes with pregnancy in hepatic fatty acid oxidation and esterification are discussed.

The intrahepatic fate of long-chain fatty acid is either to enter oxidative metabolism, resulting in the formation primarily of ketone bodies and CO_2, or to provide acyl units for the assembly of various esterified products, in particular phospholipids and triacylglycerols. The partitioning of fatty acid metabolism between oxidation and esterification varies considerably in a number of physiological states. A body of experimental findings suggests that control over this partitioning may reside mainly in the oxidative side of the esterification/oxidation branch point (McGarry *et al.*, 1973; Ontko, 1973; Ide & Ontko, 1981). The overt form of carnitine acyltransferase (CPT_1), which can be regarded as the first committed step of mitochondrial fatty acid oxidation, has particularly been implicated as a point of regulation (see review by McGarry & Foster, 1980). Hepatic CPT shows a number of noteworthy regulatory properties. Firstly, the activity is altered in the expected direction in a number of states in which fatty acid oxidation is changed (Norum, 1965; Aas & Daae, 1971; Harano *et al.*, 1972; Harper & Saggerson, 1975; Van Tol, 1975; Bremer,

1981; Saggerson *et al.*, 1982). Changes are particularly seen in the overt (CPT_1) activity (Harano *et al.*, 1972; Harper & Saggerson, 1975; Bremer, 1981; Saggerson *et al.*, 1982). At present it is unclear whether these changes reflect alterations in the amount of enzyme protein or modification of existing enzyme. Secondly, CPT_1 may be inhibited by malonyl-CoA at concentrations likely to be encountered in the liver cell and in a manner that causes considerable sigmoidicity in the kinetics with respect to [palmitoyl-CoA] (McGarry *et al.*, 1978; McGarry & Foster, 1980; Saggerson & Carpenter, 1981*b*). This effect is thought to contribute to the mechanism(s) whereby rates of hepatic fatty acid synthesis and oxidation are inversely related in a number of states (McGarry & Foster, 1980). Thirdly, the sensitivity of CPT_1 to malonyl-CoA itself appears to be under some form of control in that the inhibitory effect of this metabolite on both CPT_1 activity (Saggerson & Carpenter, 1981*a,b*; Bremer, 1981; Saggerson *et al.*, 1982) and on fatty acid oxidation by mitochondria (Cook *et al.*, 1980; Ontko & Johns, 1980; Veerkamp & Van Moerkerk, 1982) is decreased in the starved state. The hormonal or nutritional factors responsible for this change are unknown.

Abbreviations used: COT, carnitine acyltransferase activity with octanoyl-CoA; CPT, carnitine palmitoyltransferase (EC 2.3.1.21); CPT_1, the overt form of CPT.

A number of differences in hepatic fatty acid metabolism have been observed between male and female rats. The output of triacylglycerol by perfused livers from female rats exceeds that of livers from male animals (Watkins et al., 1972; Weinstein et al., 1974; Wilcox et al., 1974). A similar sex difference in hepatic triacylglycerol output is also deduced from studies in vivo (Otway & Robinson, 1967; Hernell & Johnson, 1973; Soler-Argilaga et al., 1975). Although this difference could in part be attributed to a higher rate of hepatic uptake of non-esterified fatty acid in the female (Soler-Argilaga & Heimberg, 1976; Ockner et al., 1979; Kushlan et al., 1981), additional intrahepatic factors are implicated, since, in the female, the percentage partitioning of [^{14}C]oleate between pathways of oxidation and esterification (in particular very-low-density-lipoprotein triacylglycerol formation) is more in favour of esterification in the perfused rat liver (Soler-Argilaga & Heimberg, 1976) and in isolated hepatocytes (Ockner et al., 1979). This sex difference is not attributable to any known alterations in the enzymic capacity of the esterification pathway, since there are no differences in microsomal fractions from males and females in the specific activities of fatty acyl-CoA synthetase, phosphatidate phosphohydrolase, diacylglycerol acyltransferase and (at most substrate concentrations) of glycerophosphate acyltransferase (Ockner et al., 1979). Furthermore, the incorporation of [^{14}C]oleate into total glycerolipids by microsomal fractions is not different in male and female rats (Ockner et al., 1979).

Pregnancy affects hepatic lipid metabolism in a number of respects. Hypertriglyceridaemia in the later stages of pregnancy is well documented both in the rat (Scow et al., 1964; Otway & Robinson, 1968; Hamosh et al., 1970; Wasfi et al., 1980a,b) and in women (Boyd, 1934; Peters et al., 1951; Knopp et al., 1973; Warth et al., 1975). Except when very near to term, it is thought that this hypertriglyceridaemia of pregnancy is unlikely to be due to decreased triacylglycerol clearance from the circulation (Hamosh et al., 1970; Knopp et al., 1975). Alterations in hepatic metabolism are suggested by the observation that the output of triacylglycerol is increased by perfused livers from pregnant rats (Wasfi et al., 1980a). Although hepatic uptake of oleate was unaffected by pregnancy (Wasfi et al., 1980a,b), [^{14}C]oleate metabolism was channelled preferentially into formation of esterified products rather than into oxidation in perfused livers from pregnant rats (Wasfi et al., 1980b). Increased enzymic capacity in the esterification pathway may contribute to this redirection of metabolism in pregnancy (Kalkhoff et al., 1972; Zammit, 1981). The redirection of fatty acid metabolism, however, was not observed in hepato-

cytes from pregnant rats by Whitelaw & Williamson (1977). Pregnancy is also known to result in a substantial enhancement of ketosis in starvation in man and a number of animal species (Mackay & Barnes, 1936; Gray, 1938; Fraser et al., 1938; Robertson & Thin, 1953; Bergman & Sellers, 1960; Scow et al., 1964).

The purpose of the present study was to establish the role, if any, that changes in hepatic CPT activity or its sensitivity to malonyl-CoA might play in the establishment of any of the differences.

Materials and methods

Animals

These were Sprague–Dawley rats bred in the Animal Colony at University College, London, and maintained on GR3-EK cube diet (E. Dixon and Sons, Ware, Herts, U.K.). Virgin females (average body wt. 250 g) were used at 12 weeks old. Male rats were of similar age. Pregnant rats (19–20 days gestation) were obtained by mating virgin females aged 11–12 weeks. Starvation was commenced by removal of food at 10:00 h 1 day before they were killed. All animals had drinking water throughout.

Chemicals

Palmitoyl-CoA, octanoyl-CoA, malonyl-CoA and acetyl-CoA were from International Enzymes Ltd. (Windsor, Berks, U.K.). L-Carnitine hydrochloride and bovine plasma albumin (fraction V) were from Sigma (London) Chemical Co. (Kingston-upon-Thames, Surrey, U.K.). Before use the albumin was subjected to a defatting procedure (Chen, 1967) with minor modifications (Saggerson, 1972). Oxalo-acetate was from Boehringer Corp. (London) Ltd. (Lewes, Sussex, U.K.). DL-[methyl-^{3}H]Carnitine hydrochloride was from The Radiochemical Centre (Amersham, Bucks., U.K.).

Experimental methods

Liver mitochondria were isolated as described by Saggerson (1982). In all cases the mitochondria obtained from 1 g of liver were finally stored in 4.0 ml of ice-cold 0.3 M-sucrose medium containing 10 mM-Tris/HCl (pH 7.4) and 1 mM-EGTA. The protein contents of these stock suspensions were determined by the method of Lowry et al. (1951) and contained (means, as mg of protein/ml): fed males, 4.8; fed virgin females, 5.2; starved virgin females, 6.2; pregnant, 5.3; starved pregnant, 4.9.

CPT$_1$ assays were performed within 30 min of the isolation of mitochondria as described by Saggerson et al. (1982), with 50 μl of intact mitochondria in an assay volume of 1.0 ml. In some instances overt COT activity was also measured under these conditions with 100 μM-octanoyl-CoA in place of palmitoyl-CoA. Total CPT, total COT (with 100 μM-

octanoyl-CoA) and citrate synthase (EC 4.1.3.7) activities were measured after sonication of mitochondrial preparations for 2 min at 0–2 °C as described by Saggerson et al. (1982).

Results

Fig. 1 shows that in the four tested conditions the dependence of CPT_1 activity on palmitoyl-CoA concentration was sigmoidal. A similar relationship was seen previously with mitochondria from male rats (Saggerson & Carpenter, 1981b, 1982; Saggerson et al., 1982). Table 1 shows values derived from Hill plots of the data from the individual 16 experiments that comprise Fig. 1. The Hill coefficient h and $[S]_{0.5}$ (the concentration of palmitoyl-CoA giving half-maximal CPT_1 activity) were obtained by regression analysis of the individual Hill plots, which in all cases were good-fit straight lines $(0.93 < r \leqslant 0.99)$. In the absence of malonyl-CoA, starvation and pregnancy had no effect on these parameters. In addition to the values shown in Fig. 1, CPT_1 activity was also simultaneously measured in the presence of $10 \mu M$-malonyl-CoA at each of the palmitoyl-CoA concentrations indicated in Fig. 1. The individual curves of CPT_1 activity versus [palmitoyl-CoA] from these additional experiments are not shown, but the Hill coefficients and $[S]_{0.5}$ values are given in Table 1. Malonyl-CoA had no effect on the Hill coefficient in any of the tested states, but $10 \mu M$-malonyl-CoA significantly increased the $[S]_{0.5}$ for palmitoyl-CoA by a similar extent in the fed male, fed virgin female and in the fed pregnant state. As is discussed more fully below, the effect of malonyl-CoA on the $[S]_{0.5}$ was significantly less in the starved state. As found previously (Saggerson & Carpenter, 1981b; Saggerson et al., 1982), inhibition by malonyl-CoA was seen at lower concentrations of palmitoyl-CoA, but not at the highest tested concentrations of this substrate (results not shown).

Fig. 1 indicates that CPT_1 activity relative to mitochondrial protein was quite similar in fed virgin and pregnant animals, but that the activity in starved pregnant animals was greater than that in the starved virgin rat. In Table 2, values from additional experiments to those shown in Fig. 1 are incorporated and a statistical analysis is shown at the representative palmitoyl-CoA concentrations of 40 and $100 \mu M$. Table 2 shows that CPT_1 activity was significantly lower (by approx. 25%) in the fed virgin female than in age-matched fed males. After starvation, this sex difference was no longer apparent, i.e. in percentage terms, there was a greater increase in CPT_1 after starvation in the female than in the male. These differences between the sexes were only seen when the overt CPT_1 activity was measured. No such differences in total CPT activity were

apparent. Table 2 also confirms that the CPT_1 activity measured after starvation in the pregnant state is significantly greater than that seen in the starved virgin female, i.e. there was a somewhat exaggerated response to starvation in pregnancy. In addition, pregnancy caused a small increase in total CPT activity, and this was significantly increased further by an exaggerated response to starvation. When octanoyl-CoA was used in place of palmitoyl-CoA as acyl substrate, a significant sex difference in the overt activity was again seen in the fed state together with increases in the total COT activity in the pregnant and starved pregnant states. These various changes are expressed relative to protein in the mitochondrial fraction. The same would be seen if the acyltransferase values were expressed relative to citrate synthase activity, which shows no sex differences or alteration in starvation or pregnancy (Table 2).

There was no sex difference in the inhibitory action of malonyl-CoA, i.e. in the fed male and virgin female $10 \mu M$-malonyl-CoA had a similar effect on the $[S]_{0.5}$ for palmitoyl-CoA (Table 1), and the dependence of CPT_1 activity on [malonyl-CoA] was similar (Fig. 2). In addition, in the fed state the effect of malonyl-CoA was very similar in the pregnant and virgin female animals (Table 1, Fig. 2). As shown previously (Saggerson & Carpenter, 1981a,b) with mitochondria from male rats, starvation for 24 h substantially decreased sensitivity of
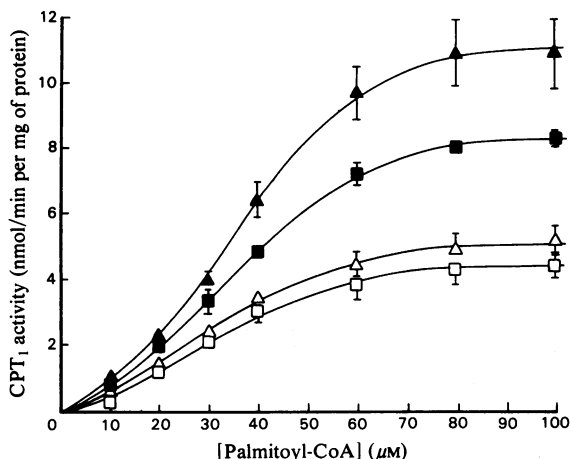

Fig. 1. *Effect of palmitoyl-CoA concentration on* CPT_1 *activity*

L-Carnitine was present throughout at $400 \mu M$. Similar measurements (results not shown) were also made in the presence of $10 \mu M$-malonyl-CoA. The values are means ± S.E.M. (indicated by bars) for four experiments in each case. □, Virgin female, fed; ■, virgin female, starved 24 h; △, pregnant, fed; ▲, pregnant, starved 24 h.

Table 1. *Effects of starvation and malonyl-CoA on kinetic properties of* CPT_1

The values are calculated from Hill plots of the data from the individual experiments summarized in Fig. 1. These are expressed as means $\pm$ S.E.M., for four experiments. The values for fed males are taken from Saggerson *et al.* (1982). *, ** indicate $P < 0.05$, <0.01 respectively for effects of starvation; †, ††, ††† indicate $P < 0.05$, <0.01, <0.001 respectively for effects of malonyl-CoA (Student's *t* test).

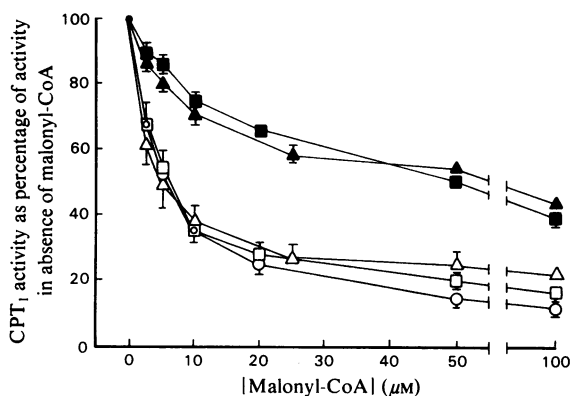| Malonyl-CoA concn.		Fed male	Fed virgin female	Starved virgin female	Fed pregnant	Starved pregnant
0	h	2.47 ± 0.11	2.50 ± 0.14	2.47 ± 0.16	2.30 ± 0.13	2.46 ± 0.09
	$[S]_{0.5}$ for palmitoyl-CoA (μM)	26.6 ± 0.3	28.2 ± 0.5	30.7 ± 1.3	26.9 ± 0.9	30.2 ± 1.8
10μM	h	2.62 ± 0.18	2.61 ± 0.21	2.53 ± 0.30	2.42 ± 0.18	2.51 ± 0.19
	$[S]_{0.5}$ for palmitoyl-CoA (μM)	42.5 ± 3.4†††	47.0 ± 2.5†††	35.8 ± 1.2**†	47.2 ± 3.4††	36.2 ± 1.3*†

Fig. 2. *Effect of malonyl-CoA concentration on* CPT_1 *activity*

L-Carnitine was present throughout at 400μM and palmitoyl-CoA at 40μM. The absolute CPT_1 activities in the absence of malonyl-CoA are given in Fig. 1. The values are means for four experiments in each case. The bars indicate S.E.M. Symbols are as for Fig. 1; also $\bigcirc$, fed males.

CPT_1 to malonyl-CoA. The same effect is seen in the virgin female rat, as evidenced by the altered inhibition curve shown in Fig. 2 and in the lesser effect of malonyl-CoA on the $[S]_{0.5}$ for palmitoyl-CoA in the starved state (Table 1). In the starved pregnant animal there was a similar alteration in the sensitivity to malonyl-CoA to that seen after starvation in virgin animals (Table 1 and Fig. 2).

Discussion

These findings suggest that alterations in sensitivity of CPT_1 to malonyl-CoA do not play a part in either the sex differences or the effects of pregnancy on hepatic fatty acid metabolism in the fed state. On the other hand, alterations in the absolute activity of CPT_1 may contribute to these changes. In the absence of any reported sex differences in the enzymic capacity of the esterification pathway (Ockner *et al.*, 1979), it is suggested that the lower CPT_1 activity in the female may assist in the preferential channelling towards esterification compared with the male. Clearly there is no sex difference in the sensitivity of CPT_1 to malonyl-CoA. At present it is unknown whether the hepatic malonyl-CoA content differs between the sexes.

Changes in the absolute activity of CPT_1 in the fed state between virgin and pregnant animals are slight, and there is no difference in sensitivity to malonyl-CoA. However, the hepatic content of malonyl-CoA is almost double in the fed pregnant rat (Zammit, 1981). The hepatic malonyl-CoA content and the rate of fatty acid synthesis are closely correlated (Guynn *et al.*, 1972; McGarry & Foster, 1980). Presumably, therefore, the increased hepatic malonyl-CoA content in the fed pregnant animal reflects an increased rate of fatty acid synthesis, which is reported as being substantially elevated in pregnancy (Fain & Scow, 1966).

Increased susceptibility of the pregnant animal to ketosis in the starved state could result from changes in rates of hepatic ketogenesis and/or changes in the rate of utilization of ketone bodies by peripheral tissues. Within the liver of the pregnant rat the following changes occur on starvation, which will contribute to acceleration of ketogenesis. Firstly, the absolute activity of CPT_1 is increased to a greater extent than is generally seen in the non-pregnant animal. Secondly, there is a substantial (14-fold) decrease in the hepatic malonyl-CoA content (compared with a 3–4-fold decrease on starvation of virgin animals) (Zammit, 1981). Thirdly, as in the non-pregnant animal, starvation decreases the sensi-

Table 2. *Enzyme activities in liver mitochondria*

In all cases these are shown as means ± S.E.M. for the numbers of experiments shown in parentheses and are expressed as nmol/min per mg of mitochondrial protein. Total CPT, total COT and citrate synthase were measured after sonication of the mitochondria at 0–2°C for 2 min. *, **, *** indicate $P<0.05$, <0.01, <0.001 for comparison of fed versus starved; †, †† indicate $P<0.05$, <0.02 for comparison of male versus female; ‡, ‡‡, ‡‡‡, ‡‡‡‡ indicate $P<0.05$, <0.02, <0.01, <0.001 for comparison of pregnant versus virgin females (Student's t test).

State	CPT_1 activity		Total CPT activity		Overt COT activity	Total COT activity	Citrate synthase activity
	With 40 μM-palmitoyl-CoA	With 100 μM-palmitoyl-CoA	With 40 μM-palmitoyl-CoA	With 100 μM-palmitoyl-CoA			
Male, fed (8)	4.1±0.3	5.6±0.4	11.1±0.6	10.3±0.6	4.2±0.3	17.4±0.9	86±3
Male, starved (4)	5.6±0.3**	9.3±0.7***	11.1±0.3	12.3±1.0	—	20.0±1.1	76±7
Virgin female, fed (6)	3.0±0.2††	4.3±0.3†	10.5±0.3	9.3±0.2	3.3±0.1††	16.2±0.5	88±3
Virgin female, starved (4)	4.9±0.1***	8.3±0.2***	10.9±0.3	10.7±0.5*	—	18.0±0.8	81±3
Pregnant, fed (6)	3.4±0.1	5.4±0.3‡	13.0±0.5‡‡‡	12.6±0.6‡‡‡‡	3.4±0.1	18.3±0.3‡‡‡	83±2
Pregnant, starved (4)	6.5±0.5***‡	11.0±0.7***‡‡	16.1±0.7**‡‡‡‡	19.2±1.2**‡‡‡‡	—	24.9±0.8***‡‡‡	81±5

tivity of CPT_1 to malonyl-CoA. Perhaps surprisingly, this decrease in sensitivity was very similar to that seen in the virgin animal. Taken together, these changes should result in a considerable activation of the ketogenic pathway when the pregnant animal is starved.

Note added in proof (Received 27 August 1982)

Between submission and acceptance of this paper, a parallel study by Robinson & Zammit (1982) has appeared. They have employed the same CPT assay as here, except that their assay had a 15-fold higher albumin/palmitoyl-CoA ratio. Under these conditions, CPT_1 activity at 40 μM-palmitoyl-CoA was approx. 10% of that reported here (i.e. 0.3 nmol/min per mg of protein) and was unaffected by pregnancy and starvation. On the other hand, under these assay conditions Robinson & Zammit (1982) found that pregnancy decreased the [malonyl-CoA]$_{0.5}$ by 50% in the fed state and increased the [malonyl-CoA]$_{0.5}$ by 4-fold in starvation. Clearly, different facets of the regulation of CPT_1 are revealed under different assay conditions.

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