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Regulation of hepatic fatty acid metabolism

The activities of mitochondrial and microsomal acyl-CoA:*sn*-glycerol 3-phosphate *O*-acyltransferase and the concentrations of malonyl-CoA, non-esterified and esterified carnitine, glycerol 3-phosphate, ketone bodies and long-chain acyl-CoA esters in livers of fed or starved pregnant, lactating and weaned rats

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1. The concentrations of malonyl-CoA, glycerol 3-phosphate, non-esterified carnitine, acid-soluble and acid-insoluble acylcarnitines, acetoacetate, 3-hydroxybutyrate and acid-insoluble acyl-CoA were measured in rapidly-frozen liver samples from fed or starved (24h) virgin, pregnant (19–20 days), lactating (2, 10–12 and 18–20 days) and weaned (for 24h, on 10th day of lactation) rats. The activities of total and *N*-ethylmaleimide-sensitive and -insensitive glycerophosphate acyltransferase (acyl-CoA:*sn*-glycerol 3-phosphate *O*-acyltransferase; EC 2.3.1.15) were also measured. 2. The concentration of malonyl-CoA was significantly higher in liver of fed pregnant, mid- and late-lactating rats than in liver of fed virgin rats. After starvation for 24h hepatic malonyl-CoA concentrations were higher in mid-lactating rats and lower in pregnant and weaned rats than in virgin animals. 3. After starvation for 24h the hepatic concentrations of glycerol 3-phosphate, ketone bodies, acid-soluble acylcarnitines and the value for the [3-hydroxybutyrate]/[acetoacetate] ratio were all highest in pregnant rats, intermediate in virgin, 2-day lactating and weaned animals and lowest in mid- and late-lactating rats. The concentrations of acid-insoluble acylcarnitines also increased most in pregnant rats, after starvation. The concentration of acid-insoluble acyl-CoA increased equally after starvation in virgin and pregnant animals but did not increase significantly in all other animals studied. 4. The total concentration of carnitine was similar in livers of fed virgin, pregnant and 2-day lactating animals but fell markedly by the 10th day of lactation and remained low in late-lactating animals. The concentration of non-esterified carnitine followed the same pattern. After starvation for 24h the hepatic concentration of non-esterified carnitine decreased significantly in virgin, pregnant and 2-day lactating animals, but remained unchanged in mid- and late-lactating or weaned animals. 5. The activities of *N*-ethylmaleimide-sensitive and -insensitive glycerophosphate acyltransferase both increased significantly in livers of mid-lactating animals. After starvation for 24h the activity of the *N*-ethylmaleimide-insensitive *O*-acyltransferase decreased in livers of virgin, pregnant and mid-lactating animals, whereas the activity of the *N*-ethylmaleimide-sensitive *O*-acyltransferase was unchanged in virgin animals but decreased markedly in livers of pregnant and lactating rats. 6. The results are discussed in relation to the importance of different metabolic parameters in the regulation of long-chain acyl-CoA metabolism in the liver.

In pregnancy and lactation increased metabolic demands are made on the mother due to the requirement for substrates either for use by the foetus(es) or for the synthesis of milk components by the mammary gland. In liver of fed pregnant and lactating rats, the metabolism of fatty acids is directed primarily towards synthesis of triacylglycerols and phospholipids (see Wasfi *et al.*, 1980; Williamson, 1980). The very-low-density lipoproteins synthesized from triacylglycerols and secreted

Abbreviations used: GPAT, glycerophosphate acyltransferase (acyl-CoA:*sn*-glycerol 3-phosphate *O*-acyltransferase; EC 2.3.1.15); NEM, *N*-ethylmaleimide.

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by the liver into the bloodstream are used as substrates of lipoprotein lipase in adipose tissue or mammary gland, depending on whether the animal is pregnant or lactating respectively. In starved rats a greater proportion of fatty acids delivered to the liver is oxidized to ketone bodies, resulting in an increase in the blood ketone-body concentration or ketonaemia (Mayes & Felts, 1967). The starved pregnant rat is more susceptible to ketonaemia, whereas rats in peak lactation are more resistant to ketonaemia than virgin animals (Hawkins & Williamson, 1972). Although these differences in the extent of ketonaemia in various physiological conditions cannot distinguish between changes in the rates of ketogenesis and/or changes in the rate of peripheral utilization of ketone bodies, studies performed *in vitro* with isolated hepatocytes from fed or starved virgin, pregnant and lactating animals have indicated that there are differences in the capacity of cells from animals in different reproductive states to synthesize ketone bodies from long-chain fatty acids (Whitelaw & Williamson, 1977). On the basis of these observations, it may be suggested that pregnancy and lactation represent successive physiological situations in which the metabolic response of the liver to starvation of the animals (for 24h) with regard to ketogenesis is more pronounced in pregnant and early-lactating rats and less pronounced in peak-lactating rats than in liver of starved virgin animals. In the present work some of the mechanisms that may underlie these differences have been investigated.

Intrahepatic regulation of ketogenesis is thought to occur at the two branch points in the ketogenic pathway, namely, at the partition of long-chain acyl-CoA between acylglycerol synthesis and acylcarnitine synthesis (and transport across the mitochondrial inner membrane) and at the partition of acetyl-CoA between synthesis of acetoacetyl-CoA and citrate (see Williamson, 1979; Zammit, 1981). The studies described in the present paper relate to regulation at the first of these metabolic branch points. Previous studies indicated that different rates of ketogenesis may be associated with (i) changes in the activities of the first enzymes in the pathways of oxidation and esterification, i.e. carnitine acyltransferase I (EC 2.3.1.21) and glycerophosphate acyltransferase (acyl-CoA:*sn*-glycerol 3-phosphate *O*-acyltransferase; EC 2.3.1.15) (Vavrečka *et al.*, 1969; Aas & Daae, 1971; Van Tol, 1974; Bates & Saggerson, 1979) and (ii) changes in the availability of the respective substrates of these enzymes, i.e. non-esterified carnitine and glycerol 3-phosphate (see Robles-Valdes *et al.*, 1976; Lund *et al.*, 1980). Whereas the maximum activity of GPAT (mitochondrial and/or microsomal) was found to change in livers of animals subjected to starvation and other experimental conditions (Bates & Saggerson, 1979), controversy existed till recently on the mode of regulation of carnitine acyltransferase I, since some workers found variations in the maximum activity of the enzyme with changes in the physiological state of the animals, whereas others could not confirm these findings (see DiMarco & Hoppel, 1975). However, it is currently accepted that changes in the maximal activity of carnitine acyltransferase I are not important in the regulation of acylcarnitine synthesis but rather that the activity of the enzyme *in vivo* is regulated by changes in the concentration of malonyl-CoA, which is a potent inhibitor of the enzyme (McGarry *et al.*, 1977). Consequently, in the present study the activities of mitochondrial and microsomal GPAT as well as the concentration of malonyl-CoA were measured in rapidly-frozen liver samples from fed or starved virgin, pregnant, lactating and weaned rats. In addition, the concentrations of the substrates of the two enzymes, i.e. glycerol 3-phosphate, non-esterified carnitine and long-chain acyl-CoA, were measured together with the concentrations of acid-soluble and acid-insoluble acylcarnitine esters, which are substrates for the carnitine:acylcarnitine carrier of the mitochondrial inner membrane. The results are discussed in relation to the possible relative importance of different regulatory mechanisms in the control of ketogenesis in livers of pregnant and lactating animals.

Materials and methods

Animals

Source, treatment and time of killing of animals were as described previously (Zammit, 1980). Weaned animals had their pups removed 24h before being killed.

Materials

Fatty acid synthase was purified from rat liver by the method of Burton *et al.* (1968). Oxoglutarate dehydrogenase was purified from pig heart as described by Garland (1974). L-[U-^{14}C]Glycerol 3-phosphate, [1-^{14}C]acetyl-CoA and $NaH^{14}CO_3$ were purchased from The Radiochemical Centre, Amersham, Bucks., U.K. All other enzymes, chemicals and reagents were purchased from Sigma (London) Chemical Co. (Poole, Dorset, U.K.).

Preparation of liver extracts

Animals were killed at 10:00h. They were anaesthetized with an intra-abdominal injection of pentobarbitone (May and Baker, Dagenham, Essex, U.K.) as described previously (Zammit, 1980). The abdominal cavity was quickly opened, the left lateral lobe of the liver was lifted and clamped *in situ* between tongs (Wollenberger *et al.*, 1960) cooled in liquid N_2. By using this method 1g samples of the liver were obtained from each rat. The liver samples

were powdered in a stainless steel mortar cooled in liquid N_2 and weighed portions were homogenized in 5 vol. of ice-cold extraction medium (see below) with a Polytron tissue disintegrator (Kinematica, Lucerne, Switzerland) fitted with probe OD10. Homogenization was carried out for two periods of 10 s at setting 5 of the instrument.

For measurement of GPAT activity the extraction medium contained 0.25 M-sucrose, 10 mM-Tris/HCl, 1 mM-EDTA and 1 mM-dithiothreitol, pH 7.4. For measurement of intermediates, tissue was extracted in 5% (w/v) $HClO_4$. Further treatment of extracts is described below.

Analytical methods

Activities of NEM-sensitive and NEM-insensitive GPAT were measured at 30°C under optimal conditions for the respective enzyme as described by Bates & Saggerson (1979).

Acid extracts were centrifuged at 2000 *g* for 5 min and the supernatants were neutralized with 3 M-$KHCO_3$/3 M-KCl to pH 7.0. The following intermediates were measured in the supernatants by methods described previously: ketone bodies (Williamson *et al.*, 1962), glycerol 3-phosphate (Hohorst, 1963), malonyl-CoA (Beynen *et al.*, 1979) and non-esterified carnitine (Cederblad & Lindstedt, 1972). Acid-soluble acylcarnitines were measured as non-esterified carnitine after hydrolysis of the supernatant with 0.5 M-KOH as described by Brass & Hoppel (1978). The acid precipitates were washed with cold 5% $HClO_4$ and water and then hydrolysed with 0.5 M-KOH containing 3 mM-dithiothreitol either at 55°C for 2 h (for hydrolysis of long-chain acylcarnitines) or for 30 min at room temperature (for hydrolysis of long-chain acyl-CoA). After hydrolysis, non-esterified carnitine was measured as above and non-esterified coenzyme-A was measured as described by Michal & Bergmeyer (1974). Preliminary experiments established that recoveries of long-chain acyl esters of CoA and carnitine were >90% in each case. Commercial preparations of CoA were standardized by the method of Garland (1974).

Statistical methods

Analysis of data was performed by Student's *t* test.

Results

The measurements of metabolite concentrations and enzyme activities were made on liver samples rapidly frozen *in situ* in animals anaesthetized with pentobarbitone. Anaesthesia was preferred to killing animals by decapitation because it was intended to detect acute effects of different physiological conditions on liver metabolism. Preservation of blood supply and oxygenation to the liver was considered particularly important in preventing rapid post-mortem changes in concentrations of metabolites that have a very high rate of turnover, e.g. malonyl-CoA (see McGarry *et al.*, 1978*c*). Instances in which the protocol used in sampling the liver is thought to have affected the results is discussed below.

Concentration of ketone bodies

In fed animals, the total concentrations of ketone bodies (acetoacetate and 3-hydroxybutyrate) in liver were very similar for all animals studied (about 0.4 μmol/g) (Table 1). The concentration ratio [3-hydroxybutyrate]/[acetoacetate] was also similar for the different types of animals (about 0.7). However, there were marked differences in the two parameters between animals at different stages in the pregnancy–lactation cycle after starvation for 24 h. After starvation for 24 h the concentration of ketones doubled in liver of virgin rats, whereas it increased 5-fold in liver of pregnant rats. For rats at different stages of lactation the increases in ketone-body concentrations were much smaller (about 50%), whereas rats weaned for 24 h after the 10th day of lactation showed a 2-fold increase in ketone-body concentration (cf. starved virgin rats). The increases in ketone-body concentration were accompanied by proportional increases in the [3-hydroxybutyrate]/[acetoacetate] ratio. Accordingly, the increase in the ratio was most pronounced in liver of starved pregnant rats (8-fold), whereas there was no increase in livers of mid- and late-lactating rats.

Concentration of malonyl-CoA

The concentration of malonyl-CoA in liver of fed virgin animals was 2.91 nmol/g fresh wt. (Table 1). This was very similar to the concentration found in isolated hepatocytes (McGarry & Foster, 1979). In pregnant and mid- and late-lactating animals the concentration was 2-fold higher. However, in 2-day lactating animals the hepatic concentration of malonyl-CoA was similar to that found in virgin rats. Weaning of 10-day lactating animals for 24 h produced no change in malonyl-CoA concentration relative to that in livers of unweaned animals. However, it altered the response of malonyl-CoA concentration to starvation (see below).

Starvation for 24 h resulted in the lowering of hepatic malonyl-CoA concentrations in all animals studied. However, the extent to which this decrease occurred varied according to the reproductive state of the animal. In virgin animals the concentration fell to 30% of that in the fed state, whereas in pregnant and weaned animals it fell to about 10% or less of that in fed animals. In livers of lactating animals the decrease in malonyl-CoA concentration was less

Table 1. *Concentrations of ketone bodies (acetoacetate plus 3-hydroxybutyrate), glycerol 3-phosphate and malonyl-CoA in frozen liver samples from fed or starved virgin, pregnant (19–20 days), lactating or weaned (for 24 h on 10th day of lactation) rats*

Values for the [3-hydroxybutyrate]/[acetoacetate] ratio are also given. Statistical significance (Student's *t* test) of the difference in concentration between fed and starved animals is indicated by $*P<0.025$, $**P<0.01$ and $***P<0.001$ and that between virgin and other animals by $\dagger P<0.025$, $\dagger\dagger P<0.01$ and $\dagger\dagger\dagger P<0.001$. The numbers of observations are given in parentheses.

		Virgin	Pregnant	Lactating			Weaned
				2-day	10–12 day	18–20 day	
Total ketone bodies (μmol/g)	Fed	0.45 ± 0.11 (6)	0.43 ± 0.12 (5)	0.43 ± 0.10 (5)	0.37 ± 0.09 (9)	0.37 ± 0.02 (3)	0.42 ± 0.06 (5)
	Starved	0.88** ± 0.16 (7)	2.01†††,*** ± 0.13 (5)	0.64†,* ± 0.12 (5)	0.56†††,** ± 0.10 (7)	0.59††,** ± 0.07 (3)	0.84*** ± 0.12 (5)
[3-Hydroxybutyrate]/ [acetoacetate] ratio	Fed	0.5	0.7	0.7	0.6	0.5	0.9
	Starved	2.3	5.3	2.2	0.7	0.6	1.6
Glycerol 3-phosphate (μmol/g)	Fed	0.50 ± 0.16 (6)	0.47 ± 0.27 (3)	0.37 ± 0.18 (4)	0.43 ± 0.13 (6)	0.40 ± 0.13 (4)	0.42 ± 0.17 (3)
	Starved	1.07*** ± 0.15 (6)	1.54†††,*** ± 0.17 (6)	0.94*** ± 0.18 (5)	0.52††† ± 0.20 (7)	0.62†††,* ± 0.06 (3)	0.77††,** ± 0.14 (6)
Malonyl-CoA (nmol/g)	Fed	2.91 ± 0.58 (3)	5.58† ± 0.97 (3)	2.81 ± 0.34 (4)	4.62 ± 1.64 (5)	6.58† ± 1.11 (3)	4.74 ± 1.96 (4)
	Starved	0.82** ± 0.09 (3)	0.43†,*** ± 0.17 (4)	0.47††,*** ± 0.14 (4)	1.55†,** ± 0.58 (6)	1.14*** ± 0.26 (3)	0.39††,*** ± 0.18 (5)

pronounced. Thus, in starved mid-lactating rats the hepatic concentration of malonyl-CoA was 2-fold higher than that in liver of starved virgin rats (1.55 and 0.82 nmol/g for lactating and virgin animals respectively).

Concentration of glycerol 3-phosphate

In fed animals the hepatic concentration of glycerol 3-phosphate was very similar for all the animals studied (about 0.4 μmol/g) (Table 1). Starvation (24 h) resulted in a 2-fold increase in concentration in virgin rats and a 3-fold increase in pregnant animals. In lactating animals there was little or no increase in glycerol 3-phosphate concentration after starvation. Thus the hepatic concentration of glycerol 3-phosphate in 10–12-day-lactating rats was significantly lower than in liver of starved virgin rats. Conversely, the concentration in liver of starved pregnant rats was 50% higher than that in starved virgin rats.

Concentration of non-esterified carnitine and acyl-carnitine esters

The concentration of non-esterified carnitine was very similar in livers of fed virgin, pregnant and 2-day-lactating rats, but was markedly lower in livers of 10–12-day-lactating animals (Table 2). There was a small, but statistically insignificant, increase in the concentration of non-esterified carnitine in liver of weaned and late-lactating animals. After starvation for 24 h there was a significant decrease in the non-esterified carnitine concentration in liver of virgin animals. In livers of pregnant and 2-day-lactating rats this decrease was much more pronounced (Table 2), whereas in weaned and in 10- and 20-day lactating animals starvation had no significant effect on the concentration of non-esterified carnitine.

The concentration of acid-soluble acylcarnitines (a measure of short- and medium-chain acylcarnitines; see Pearson & Tubbs, 1967) increased in the liver of all animals studied after starvation for 24 h. This increase was most pronounced in pregnant and weaned animals (3-fold) and smaller in 10–12-day-lactating animals (40%). As a result, the concentration of acid-soluble acylcarnitines in liver of starved pregnant rats was twice as high as that in liver of starved virgin animals, whereas it was significantly lower in liver of starved 10–12-day-lactating rats.

The concentration of acid-insoluble (long-chain) acylcarnitine esters in livers of fed animals ranged from 3.8 (weaned and 18–20-day-lactating animals) to about 9.0 nmol/g (pregnant and 2-day-lactating animals) (Table 2). After starvation for 24 h the concentration increased about 2-fold in all the animals except in pregnant rats in which the increase was 4-fold (to 33.9 nmol/g) (Table 2). These values

Table 2. *Concentration of non-esterified carnitine, acid-soluble and acid-insoluble acylcarnitine esters and acid-insoluble acyl-coenzyme A esters in frozen liver samples from fed or starved virgin, pregnant (19–20 day) lactating or 24 h-weaned (on 10th day of lactation) rats*

Values (nmol/g wet wt.) are given as means ± S.E.M. Statistical significance (Student's *t* test) of the difference in concentration between fed and starved animals is indicated by $*P<0.05$, $**P<0.01$ and $***P<0.001$ and that between virgin and other animals by $\dagger P<0.05$, $\dagger\dagger P<0.01$ and $\dagger\dagger\dagger P<0.001$. The numbers of observations are given in parentheses.

		Concentration (nmol/g wet wt.)					
				Lactating			
		Virgin	Pregnant	2 day	10–12 day	18–20 day	Weaned
Non-esterified carnitine	Fed	329 ± 35 (4)	393 ± 57 (5)	367 ± 30 (3)	128†† ± 62 (4)	213† ± 69 (3)	213 ± 74 (3)
	Starved	214* ± 69 (4)	181*** ± 30 (3)	173** ± 38 (3)	160 ± 24 (4)	137 ± 53 (3)	155 ± 38 (5)
Acid-soluble acylcarnitine	Fed	159 ± 30 (4)	158 ± 29 (5)	196 ± 41 (3)	114†† ± 19 (4)	93†† ± 21 (4)	90†† ± 25 (3)
	Starved	229* ± 36 (4)	481†††, *** ± 30 (3)	363††,* ± 50 (3)	165†,* ± 22 (4)	192* ± 48 (3)	260*** ± 49 (5)
Acid-insoluble acylcarnitine	Fed	6.4 ± 1.8 (6)	9.5 ± 2.4 (4)	8.4 ± 2.8 (5)	5.2 ± 1.8 (4)	3.8† ± 0.8 (3)	4.2 ± 0.9 (5)
	Starved	13.3** ± 4.8 (10)	33.9†††, *** ± 6.7 (4)	13.3* ± 3.6 (6)	8.3* ± 1.8 (6)	10.7** ± 2.9 (3)	10.7** ± 3.6 (5)
Acid-insoluble acyl-CoA	Fed	57.2 ± 5.3 (3)	76.3†† ± 4.1 (3)	42.7 ± 6.6 (3)	68.4 ± 5.3 (3)	65.3 ± 7.8 (3)	54.1 ± 7.2 (3)
	Starved	107.5*** ± 18.5 (3)	104.8*** ± 15.8 (4)	54.9††† ± 4.3 (3)	71.7††† ± 5.8 (3)	72.7††† ± 6.3 (3)	62.1††† ± 8.1 (3)

were, generally, lower than those reported by Pearson & Tubbs (1967) and Brass & Hoppel (1978), who obtained liver samples after decapitation of the animals. Values very similar to those reported here were found by Pearson & Tubbs (1967) when liver was sampled in pentobarbitone-anaesthetized rats.

Concentration of long-chain acyl-CoA

In fed animals only the concentration of long-chain acyl-CoA in liver of pregnant rats was significantly different (50% higher) than that in all animals studied (Table 2). After starvation for 24 h the concentration increased to similar values in livers of virgin and pregnant rats but did not increase significantly in livers of lactating and weaned animals. Consequently, the concentration of long-chain acyl-CoA in livers of starved virgin and pregnant rats was 40–50% higher than in livers of starved lactating and weaned animals.

Activities of NEM-sensitive and NEM-insensitive GPAT

The total activity of GPAT (NEM-sensitive plus NEM-insensitive) in livers of mid- and late-lactating animals was significantly higher in liver of mid- and late-lactating rats than that in virgin rats when expressed either in terms of liver protein content, fresh weight, or per whole liver. Weaning (24 h) of 10-day lactating animals resulted in a 55% decrease in total activity (results not shown).

The NEM-sensitive and NEM-insensitive enzyme activities (microsomal and mitochondrial respectively; see Bates & Saggerson, 1979), when expressed in terms of whole-liver protein, responded differently to the nutritional and reproductive states of the animal (Table 3). The microsomal activity was 60% higher in liver of pregnant and mid-lactating rats than in liver of virgin animals. However, the values returned to those found in virgin animals after starvation for 24 h. Starvation of virgin animals produced no change in microsomal GPAT activity (cf. Bates & Saggerson, 1979), but resulted in significant decreases in activity in livers of pregnant and lactating animals.

Mitochondrial GPAT activity was 40% higher in livers of mid- and late-lactating animals relative to the activity in fed virgin animals. Weaning of 10-day-lactating animals restored the activity to values similar to those in liver of virgin animals. Starvation resulted in a 30% decrease in mitochondrial GPAT activity in virgin rats (cf. Bates & Saggerson, 1979), but in much smaller decreases in activity in livers of lactating animals. Thus the activity of mitochondrial GPAT was significantly higher in liver of starved mid-lactating rats than of starved virgin animals (Table 3).

Table 3. *Activities of NEM-sensitive and NEM-insensitive sn-glycerol 3-phosphate acyltransferase (nmol/min per mg of protein at 30°C) in extracts of rapidly frozen liver samples (see the Materials and methods section) from fed or starved virgin, pregnant (19–20 day), lactating and 24 h-weaned (on 10th day of lactation) rats*

The values are means ± S.E.M. The numbers of determinations for each value are given in parentheses. Statistical significance (Student's *t* test) of the difference in activity between fed and starved animals is indicated by $*P<0.025$, $**P<0.01$ and $***P<0.001$, and that between virgin and other animals by $\dagger P<0.025$, $\ddagger P<0.05$ and $\dagger\dagger\dagger P<0.001$.

				Lactating			
		Virgin	Pregnant	2 day	10–12 day	18–12 day	Weaned
NEM-insensitive GPAT activity	Fed	0.72 ± 0.10 (6)	0.66 ± 0.07 (4)	0.70 ± 0.06 (4)	0.99†† ± 0.05 (9)	0.98†† ± 0.13 (6)	0.62 ± 0.06 (4)
	Starved	0.50** ± 0.07 (3)	0.48* ± 0.10 (3)	0.67† ± 0.10 (4)	0.77††,*** ± 0.05 (3)	—	—
NEM-sensitive GPAT activity	Fed	0.31 ± 0.09 (9)	0.50††† ± 0.04 (4)	0.37 ± 0.07 (6)	0.50††† ± 0.09 (9)	0.35 ± 0.06 (5)	0.20 ± 0.07 (4)
	Starved	0.32 ± 0.04 (3)	0.17††,*** ± 0.03 (3)	0.21** ± 0.06 (4)	0.21†,*** ± 0.03 (3)	—	—

Discussion

Effects of starvation on hepatic fatty acid oxidation

In the present study no direct measurements of rates of hepatic fatty acid oxidation were made. (For direct measurements of oxidation and/or ketogenesis in liver preparations from pregnant and lactating animals see Whitelaw & Williamson, 1977; Wasfi *et al.*, 1980.) However, some of the data obtained, namely the concentrations of ketone bodies, the [3-hydroxybutyrate]/[acetoacetate] ratio and the concentrations of short- and medium-chain acylcarnitines, may be used as qualitative indicators of the relative rates of hepatic fatty acid oxidation in livers of animals in the various metabolic and reproductive states.

In general, the results suggested that in the fed state the rate of ketone body formation was low in livers of all animals studied. After starvation of the animals for 24 h, the livers of late pregnant rats oxidized fatty acids most rapidly, whereas the rate of oxidation in virgin and early lactating and weaned rats was higher than that in livers of mid- and late-lactating animals. All three parameters (see above) were highest in livers of starved pregnant rats, intermediate in livers of early lactating, weaned and virgin animals and lowest in mid- and late-lactating rats.

Mechanisms of regulation of long-chain acyl-CoA metabolism

(*a*) *Changes in the availability of substrates.* The concentration of long-chain acyl-CoA increased only in livers of virgin and pregnant rats after starvation for 24 h. Since the concentration of acyl-CoA in the liver is likely to reflect the balance between the rate of delivery of fatty acids to the liver and their rate of utilization for esterification and oxidation it is possible that the failure of long-chain acyl-CoA concentration to rise after starvation in lactating animals may have resulted either from a continued high rate of triacylglycerol synthesis in livers of these animals or from an inability of adipose tissue to increase lipolysis after starvation. The altered response of long-chain acyl-CoA concentrations after starvation in lactating animals may be important in view of the fact that long-chain acyl-CoA esters affect certain metabolic processes and activities of enzymes (e.g. the tricarboxylate carrier and acetyl-CoA carboxylase; see Halperin *et al.*, 1972; Goodridge, 1972).

Results of previous work suggested that the availability of glycerol 3-phosphate and of non-esterified carnitine in the liver could affect the rate of long-chain acyl-CoA esterification and oxidation respectively (Wieland & Matschinsky, 1962; McGarry *et al.*, 1975; Lund *et al.*, 1980). In the present study the concentration of glycerol 3-phos-

phate increased after 24h-starvation in the liver of all animals studied with the exception of rats in peak lactation. Plots of hepatic glycerol 3-phosphate concentration against ketone-body concentration, [3-hydroxybutyrate]/[acetoacetate] ratio and short-chain acylcarnitine concentration in livers of starved animals all yielded straight lines with regression coefficients of 0.91, 0.98 and 0.88 respectively (Fig. 1). This indicated that glycerol 3-phosphate concentration varied proportionately with the rate at which the liver oxidized fatty acids, as would be expected from increased delivery of glycerol to the liver from adipose tissue under ketogenic conditions. Therefore it is unlikely that glycerol 3-phosphate availability limited the rate of utilization of fatty acids for triacylglycerol synthesis in any of the conditions studied.

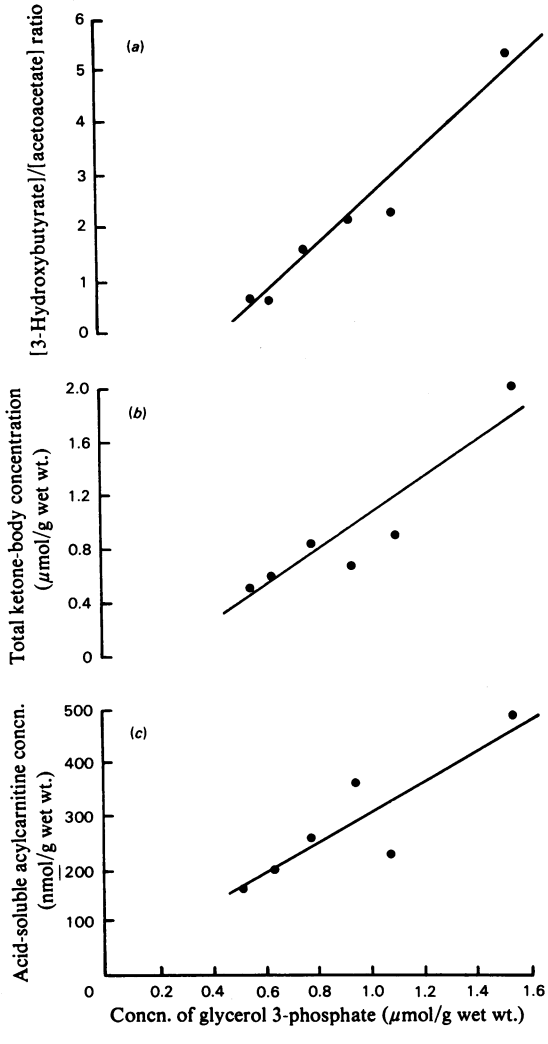

Fig. 1. *Relation between concentrations of glycerol 3-phosphate and (a) [3-hydroxybutyrate]/[acetoacetate] ratio, (b) total ketone-body concentration and (c) acid-soluble acylcarnitine concentrations in rapidly frozen liver samples from starved virgin, pregnant, lactating or weaned animals*

The straight lines were fitted using the least-squares method. The correlation coefficients were (*a*) 0.98, (*b*) 0.91 and (*c*) 0.88.

It is also unlikely that the concentration of non-esterified carnitine had a direct (mass-action) effect on the synthesis of long-chain acylcarnitines as suggested previously for pregnant and early lactating rats (Robles-Valdes *et al.*, 1976; McGarry *et al.*, 1978*b*). The concentration of non-esterified carnitine was similar in livers of fed virgin, pregnant and 2-day lactating rats and fell significantly after starvation of animals for 24h in these physiological states. Both these sets of observations were at variance with the observations of McGarry *et al.* (1978*b*) probably due to differences in the fat content of the diet. The observation that changes consistent with increased rates of fatty acid oxidation occurred in livers of starved virgin, early lactating and, especially, pregnant animals in spite of a decrease in the non-esterified carnitine content of the liver raises the question as to whether changes in non-esterified carnitine concentration are central to the increased diversion of long-chain acyl-CoA metabolism towards oxidation under ketogenic conditions. It is also noteworthy that although in the liver of starved peak-lactating animals the concentration of non-esterified carnitine was similar to that of starved virgin and pregnant rats, the rate of ketogenesis in liver of lactating rats was much lower. Changes in the concentration of short-chain acylcarnitine esters are more likely to be directly related to the ketogenic capacity of the liver (cf. Brass & Hoppel, 1980).

(*b*) *Changes in the activities of GPAT and the concentration of malonyl-CoA.* In the present study a good degree of correlation was found (see Fig. 2) between GPAT activity in the liver of fed or starved animals and previously reported rates of synthesis of esterified products from oleate by isolated hepatocytes from animals in the various reproductive states studied (Whitelaw & Williamson, 1977). This observation suggests that changes in the activity of GPAT may be involved in the regulation of partition of long-chain acyl-CoA between esterification and oxidation.

The activities of mitochondrial and microsomal GPAT in the liver have previously been shown to respond differently to changes in the nutritional status of rats and to acute hormonal stimuli in perfused livers (see Bates & Saggerson, 1979). Both forms of the enzyme increased in activity in mid- and late-lactation. The increase in the activity of the microsonal enzyme preceded that of the mito-

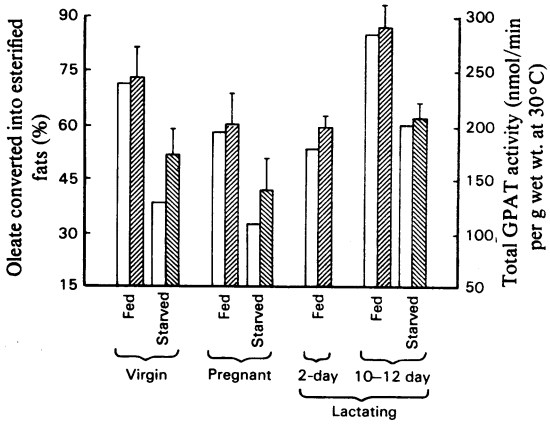

Fig. 2. *Relation between the total activity of GPAT (nmol/min per g wet wt. at 30°C) in rapidly frozen liver samples and the percentage of oleate taken up by hepatocytes converted into esterified products*

The data on esterification are taken from Whitelaw & Williamson (1977); no results for esterification in hepatocytes from starved 2-day-lactating rats are available. The height of cross-hatched areas indicates the mean GPAT activity and the vertical bars indicate 1 S.D.

chondrial enzyme and, in general, the fractional changes in the activity of the microsomal enzyme were greater (especially after starvation in pregnant and lactating animals). Thus, although the maximal activity of the mitochondrial enzyme *in vitro* was about twice that of the microsomal enzyme, it is difficult to suggest roles or quantitative assessments of the relative importance of the two enzymes in the increased rate of triacylglycerol synthesis in the liver of fed pregnant and lactating rats since the two enzymes may have access to different pools of long-chain acyl-CoA in the cell.

The activity of carnitine acyltransferase I *in vivo* is probably determined by the cytosolic concentration of malonyl-CoA, which is a potent inhibitor of the enzyme with a K_i of 2 μM *in vitro* (McGarry *et al.*, 1978*a*). The concentration of malonyl-CoA in the liver was also found to be inversely related to the rate of hepatic ketogenesis with an apparent K_i of 2 nmol/g wet wt. of liver (Cook *et al.*, 1978). The concentrations of malonyl-CoA measured in livers of fed animals in this study were higher than the K_i for the enzyme *in vitro*, and it is reasonable to assume that in liver of fed animals the enzyme is substantially inhibited in virgin and early lactating rats and even more strongly inhibited in liver of pregnant and mid- and late-lactating animals. Therefore, the higher concentrations of malonyl-CoA during pregnancy and lactation may contribute towards a greater proportion of fatty acids being diverted to triacylglycerol synthesis. Of particular interest were the differences in the response of the malonyl-CoA concentration in livers of animals in the various reproductive states after starvation for 24 h. In livers of starved pregnant and weaned animals the concentrations fell to about 0.4 nmol/g, which were similar to the lowest concentrations previously found in isolated liver cells in which fatty acid synthesis was negligible (McGarry & Foster, 1979). However, in livers of animals in peak lactation (10–12 days) the concentration of malonyl-CoA after starvation was about 4-fold greater (1.55 nmol/g). This suggested that during lactation substantial fatty acid synthesis and inhibition of carnitine acyltransferase I occurred even after starvation for 24 h. The differences in the effect of malonyl-CoA may be even more accentuated than suggested by the concentrations observed in the light of recent reports that the sensitivity of the rate of ketogenesis in isolated liver mitochondria to inhibition by malonyl-CoA may vary with the nutritional state of the animal (Cook *et al.*, 1980; Ontko & Johns, 1980).

General conclusions

The results presented in this paper suggest that changes in the relative utilization of long-chain acyl-CoA for synthesis of esterified products or for oxidation in liver of rats in different reproductive and/or nutritional states may be due to a combination of regulatory mechanisms. Of primary importance appear to be (i) the changes in activities of the enzymes that catalyse the first reactions of the pathway of esterification from glycerol 3-phosphate and of long-chain acylcarnitine synthesis and (ii) modulation of the concentration of short- and medium-chain acylcarnitine content of the liver. Changes in the availability of the respective initial substrates (namely, glycerol 3-phosphate and non-esterified carnitine) do not appear to be directly related to the rates of the two pathways. In conjunction with the changes in the ability of mitochondria to oxidize long-chain acylcarnitine esters (Zammit, 1980) these regulatory mechanisms may account for most of the observed differences in hepatic fatty acid metabolism in the various reproductive states.

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