Relationship between acid-soluble carnitine and coenzyme A pools *in vivo*

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The relationship between the acid-soluble carnitine and coenzyme A pools was studied in fed and 24-h-starved rats after carnitine administration. Carnitine given by intravenous injection at a dose of 60 μ mol/100 g body wt. was integrated into the animal's endogenous carnitine pool. Large amounts of acylcarnitines appeared in the plasma and liver within 5 min of carnitine injection. Differences in acid-soluble acylcarnitine concentrations were observed between fed and starved rats after injection and reflected the acylcarnitine/carnitine relationship seen in the endogenous carnitine pool of the two metabolic states. Thus, a larger acylcarnitine production was seen in starved animals and indicated a greater source of accessible acyl-CoA molecules. In addition to changes in the amount of acylcarnitines present, the specific acyl groups present also varied between groups of animals. Acetylcarnitine made up 37 and 53% of liver acid-soluble acylcarnitines in uninjected fed and starved animals respectively. At 5 min after carnitine injection hepatic acid-soluble acylcarnitines were 41 and 73% in the form of acetylcarnitine in fed and starved rats respectively. Despite these large changes in carnitine and acylcarnitines, no changes were observed in plasma non-esterified fatty acid or β -hydroxybutyrate concentrations in either fed or starved rats. Additionally, measurement of acetyl-CoA, coenzyme A, total acid-soluble CoA and acid-insoluble CoA demonstrated that the hepatic CoA pool was resistant to carnitine-induced changes. This lack of change in the hepatic CoA pool or ketone-body production while acyl groups are shunted from acyl-CoA molecules to acylcarnitines suggests a low flux through the carnitine pool compared with the CoA pool. These results support the concept that the carnitine/acid-soluble acylcarnitine pool reflects changes in, rather than inducing changes in, the hepatic CoA/acetyl-CoA pool.

Changes in carnitine metabolism with alterations in metabolic state have been demonstrated in animal (Pearson & Tubbs, 1964, 1967; Bohmer *et al.*, 1966; Bohmer, 1967; Snoswell & Koundakjian, 1972; Snoswell & McIntosh, 1974; McGarry *et al.*, 1975; McGarry & Foster, 1977; Brass & Hoppel, 1978*a*) and human (Maebashi *et al.*, 1976, 1977*a,b*; Frohlich *et al.*, 1978; Genuth & Hoppel, 1979; Hoppel & Genuth, 1980) experimental models. Two types of changes have been described: (1) an increase or decrease in the amount of total carnitine present in a tissue or fluid compartment and (2) a change in the proportion of the total carnitine present as acylcarnitines. These acylcarnitines consist of both acid-soluble and acid-insoluble acylcarnitines. The acid-insoluble acylcarnitines consist largely of long-chain acylcarnitines and represent the intermediates of carnitine-mediated fatty acid transport into mitochondria. However, acid-soluble

acylcarnitines, for which no specific role has been defined, are present in almost all tissues at higher concentrations than the acid-insoluble acylcarnitines. Since acylcarnitines are produced by the reversible transfer of an acyl group from acyl-CoA to carnitine, a change in acylcarnitine concentration may arise from, or cause changes in, the concentration of acyl-CoA. For example, acetylcarnitine formation is at the expense of acetyl-CoA. Any change in acyl-CoA concentrations may have dramatic effects on the activity of many metabolic pathways.

Several experiments have suggested that carnitine administration may cause changes in hepatic metabolism. Carnitine has been found to be anti-ketogenic (Broekhoysen *et al.*, 1965; Gravina & Gravina-Sanvitale, 1969), hypolipidaemic (Fujii *et al.*, 1972; Hosein & Bexton, 1975; Maebashi *et al.*, 1978; Bougneres *et al.*, 1979) and hypoglycaemic

(Fujii *et al.*, 1972) *in vivo*, but it increases ketone-body production from the perfused liver system (McGarry *et al.*, 1975) and isolated hepatocytes (Christiansen *et al.*, 1976). Mechanisms for these effects have not been elucidated, but the interaction of carnitine with the hepatic CoA pool provides potential mechanisms for these observations. However, observations *in vivo* to date (Seim & Strack, 1977) and recent work *in vitro* (Brass & Hoppel, 1980; Markwell *et al.*, 1973) have suggested that the capacity of liver mitochondria to transfer acetyl and other acyl groups from intramitochondrial CoA to carnitine is probably small compared with the total acyl-group flux.

To further study this interaction between the carnitine and coenzyme A pools, (–)-carnitine was administered by intravenous injection to fed and starved rats. After carnitine administration, no change in plasma β -hydroxybutyrate or non-esterified fatty acid concentration was seen in either metabolic state. Large amounts of acid-soluble acylcarnitines were produced indicating that the exogenous carnitine had access to an acyl-CoA pool. Despite those increases in acylcarnitines, only small changes were seen in the hepatic-CoA pool. This suggests that the high flux of acyl groups through the CoA pool prevents any major change in the pool as a small percentage of the acyl groups are shunted to acylcarnitine formation.

Experimental

Animals

Male Sprague–Dawley rats (175–250 g) were used in all experiments. Rats were permitted Purina rat chow and water *ad libitum*. Experimental protocols involving fed animals were initiated within 2 h after the lights in the animal room were turned on (lights were automatically controlled with a 14 h on–10 h off cycle). Periods of starvation were begun during this same 2 h by removal of food from cages. Starved rats were kept in individual wire-bottom cages and allowed water *ad libitum*.

Administration of exogenous carnitine was performed by intravenous (tail) injections. All solutions for injection were neutralized. Injection volumes were in the range 0.3–0.5 ml. Iso-osmotic solutions were not used because of the larger injection volumes they would have required. Control experiments (equal doses given as hyper-osmotic and iso-osmotic solutions) demonstrated that such hyperosmotic injections did not cause changes in any of the variables of interest. Control animals received saline (1.8% NaCl) injections.

Rats were killed by decapitation and blood was collected in heparin. The abdomen was then opened and liver tissue was rapidly frozen *in situ* with

aluminium blocks cooled in solid CO₂/acetone (elapsed time 20–30 s). The effect of this delay on the hepatic content of metabolic intermediates, particularly acetyl-CoA, was of great concern. Data from the literature indicate that such a delay in freezing might result in a 20% decrease (Pearson & Tubbs, 1967) or a 30% increase (Lumbers *et al.*, 1969) in acetyl-CoA. No satisfactory solution to this problem exists in the rat model. Specifically, in unpublished experiments we have used rats anaesthetized with diethyl ether as suggested by Lumbers *et al.* (1969) and confirmed a 17% lower content of acetyl-CoA in livers obtained from fed anaesthetized animals compared with decapitation. However, diethyl ether caused fundamental changes in other metabolic parameters (i.e. a 38% decrease in plasma non-esterified fatty acids in starved animals) without altering the pattern of changes in hepatic acetyl-CoA between fed and starved animals as reported in the present paper with decapitation. The use of phenobarbital as well as other methods of killing were without clear benefit over the decapitation procedure. Thus, although the hepatic content of acetyl-CoA, and to a lesser extent other CoA and carnitine derivatives, may be as much as 20% lower than those reported in the present paper, the conclusions based on the patterns of changes rather than absolute magnitudes would not be altered.

By using ¹²⁵I-labelled albumin, it was found that liver samples from fed rats contained 0.0655 ± 0.018 ml (mean $\pm$ s.d.) of plasma/g, and samples from 24-h-starved rats 0.090 ± 0.009 ml of plasma/g. These values and haematocrits at 0.45 and 0.50 in fed and 24-h-starved rats respectively permitted correction of all measurements for blood trapped in frozen liver samples by using the following equation (written for fed animals):

True content =

$$\frac{\text{nmol/g measured} - (0.0655 \times \text{nmol/ml of plasma})}{1 - (0.0655/0.45)}$$

Assays

Carnitine, acid-soluble acylcarnitines and acid-insoluble acylcarnitines were measured by a radioisotope method as previously described (Brass & Hoppel, 1978a). Acetylcarnitine and acyl-CoA were determined by a method similar to that of Decker (1974). Samples (neutralized HClO₄ extract plus water, totalling 0.5 ml) were added to 0.50 ml of a working solution (0.4 M-Tris buffer, pH 8.1, 0.1 mg of coenzyme A/ml, 1 mM-EDTA, 1.1 mM-malate and 0.05 mM-NAD⁺). Malate dehydrogenase (5 units) was added to permit baseline formation of oxaloacetate and NADH. When the fluorescence had stabilized, citrate synthase (1.1 units) was added. After equilibration, the increase in fluorescence provided a measure of acetyl-CoA. Addition of

carnitine acetyltransferase (2 units) at this point converted all the acetylcarnitine into acetyl-CoA, which then caused a second increase in fluorescence and provided a measure of acetylcarnitine. In all cases 1 unit of enzyme activity is the amount required to convert 1 μmol of substrate/min.

The recycling assay for coenzyme A developed by Allred & Guy (1969) was used in neutralized HClO_4 extracts (to provide a measure of the sum of coenzyme and acetyl-CoA), on neutralized HClO_4 extracts after alkaline hydrolysis (a measure of total acid-soluble CoA) and on the HClO_4 -insoluble pellet after alkaline hydrolysis (as a measure of acid-insoluble acyl-CoA). Coenzyme A concentrations in standard solutions were determined by the end-point-u.v.-absorbance method (Michal & Bergmeyer, 1974).

Plasma non-esterified fatty acids and β -hydroxybutyrate were determined as previously described (Brass & Hoppel, 1978a).

Reagents

Chemical and solvents used were of reagent grade. Malate dehydrogenase, citrate synthase, phosphotransacetylase and carnitine acetyltransferase were purchased from Boehringer (Mannheim) Corp., Indianapolis, IN, U.S.A. β -Hydroxybutyrate dehydrogenase was purchased from Sigma Chemical Co., St. Louis, MO., U.S.A. (–)-Carnitine chloride was a gift from Otsuka Pharmaceuticals, Osaka, Japan.

Results

After injection of carnitine (60 $\mu\text{mol}/100\text{g}$ body wt.) there was a large rapid increase in plasma carnitine concentration in both fed and 24-h-starved animals (Tables 1 and 2). After the immediate increase, the plasma concentration began to decrease rapidly towards normal. At 30 min after injection, the plasma concentration was only approx. 15% of peak concentrations. This decrease in concentration continued at a slower rate during the remainder of the 2 h time course. After 2 h, the plasma carnitine concentration was 3.5-fold increased in both starved and fed animals compared with controls.

The disappearance of carnitine from the plasma compartment probably consists of both distribution of the carnitine into extracellular fluid and tissues, as well as irreversible elimination of carnitine. Since both these processes may be regulated, it was of interest to quantify the elimination of carnitine from the plasma compartment. In both fed and fasted animals, the time course, when plotted on a semi-logarithmic scale, could be divided into two regions, an initial 30 min period of rapid elimination followed by a period of slower carnitine disappear-

ance from the plasma. When the initial 30 min period was approximated by first-order kinetics, an elimination rate constant from the plasma could be estimated in fed and 24-h-starved animals. The data from the first 30 min of the time course was fitted to a straight line by the method of least squares. From these calculations the elimination constants were estimated to be 0.0245 min^{-1} and 0.0252 min^{-1} in fed and starved animals respectively. These rate constants correspond to half-lives of 28 min in the fed and 24 min in the starved animals. While this represents only the simplest approximation of the elimination kinetics, it suggests that there is no difference in carnitine elimination kinetics from the plasma between fed and 24-h-starved rats.

The changes in plasma acid-soluble acylcarnitines are also shown in Tables 1 and 2. In both fed and starved animals there was a peak in plasma acid-soluble acylcarnitine concentration during the first 30 min after injection. After this peak in the fed animals there was a slow decrease ($P < 0.01$ at 10 min vs. 120 min) in concentration during the remainder of the time course. In contrast with starved rats, the plasma acid-soluble acylcarnitine concentration remained near peak values during the remainder of the 2 h time course.

The ratio of acid-soluble acylcarnitine to carnitine concentration in the plasma changed markedly during the 2 h after carnitine injection. Consistent with previous observations (Brass & Hoppel, 1978a) this ratio was significantly increased ($P < 0.01$) in the starved animals compared with fed controls before injection (1.71 versus 0.66). A sharp decrease ($P < 0.01$) in the ratio was seen in both fed and starved animals 5 min after injection, followed by a slow increase in the proportion of the total acid-soluble carnitine present as acylcarnitines. After 1 h the ratio was very close to the values seen in the control animals. At 2 h the ratio was increased above that seen in controls, but only significantly so in the starved animals ($P < 0.01$ in starved animals, $0.025 > P > 0.01$ for fed animals) reflecting the more sustained increase in plasma acid-soluble acylcarnitines observed in these animals.

Acid-insoluble acylcarnitines were increased in the plasma 2 h after injections in both fed (5.55 μM vs. 11.84 μM) and starved (5.51 μM vs. 17.10 μM) animals.

Table 3 shows the liver content of carnitine, acid-soluble acylcarnitines, acid-insoluble acylcarnitines and total carnitine in fed animals injected with carnitine (60 $\mu\text{mol}/100\text{g}$ body wt.). After reaching a peak between 10 and 30 min, the carnitine content decreased slowly and was still 4-fold elevated after 120 min. Acid-soluble acylcarnitines also increased rapidly, following a time course similar to that seen for carnitine. As previously reported (Brass & Hoppel, 1978b), acid-insoluble carnitines were also

Table 1. *Plasma acid-soluble carnitines after carnitine administration to fed rats*

(—)Carnitine was given to fed rats at a dose of 60 $\mu\text{mol}/100\text{g}$ body wt. by intravenous injection. At the times indicated animals were killed and plasma samples were collected. Carnitine, acid-soluble acylcarnitines, non-esterified fatty acids (NEFA) and β -hydroxybutyrate (β -OH) were determined as described in the Experimental section. Values are means $\pm$ s.d. Numbers of animals used for determinations of [NEFA] and [β -OH] are shown in parentheses when different from the numbers for carnitine determinations.

Time (min)	n	[Carnitine] (μM)	[Acid-soluble acylcarnitines] (μM)	[Acid-soluble acylcarnitines]/	[NEFA] (mM)	[β -OH] (mM)
				[carnitine]		
0	16	29 $\pm$ 6	19 $\pm$ 8	0.66 $\pm$ 0.21	0.24 $\pm$ 0.06 (15)	0.14 $\pm$ 0.04
1	5	2830 $\pm$ 200	360 $\pm$ 100	0.13 $\pm$ 0.03	0.23 $\pm$ 0.08	0.14 $\pm$ 0.01
5	7	1540 $\pm$ 420	60 $\pm$ 70	0.04 $\pm$ 0.04	0.26 $\pm$ 0.06	0.15 $\pm$ 0.03
10	3	1200 $\pm$ 120	207 $\pm$ 97	0.18 $\pm$ 0.09	0.23 $\pm$ 0.04	0.17 $\pm$ 0.05
20	3	790 $\pm$ 130	140 $\pm$ 60	0.18 $\pm$ 0.07	0.16 $\pm$ 0.03	0.10 $\pm$ 0.02
30	6	460 $\pm$ 60	100 $\pm$ 40	0.22 $\pm$ 0.07	0.23 $\pm$ 0.06 (5)	0.12 $\pm$ 0.06
60	4	240 $\pm$ 20	110 $\pm$ 40	0.48 $\pm$ 0.13	0.28 $\pm$ 0.08	0.17 $\pm$ 0.05
120	6	100 $\pm$ 23	75 $\pm$ 17	0.76 $\pm$ 0.13	0.19 $\pm$ 0.03	0.10 $\pm$ 0.02

Table 2. *Plasma acid-soluble carnitines after carnitine administration to 24-h-starved rats*

(—)Carnitine was given to 24-h-starved rats at a dose of 60 $\mu\text{mol}/100\text{g}$ body wt. by intravenous injection. At the times indicated animals were killed and plasma samples were collected. Carnitine, acid-soluble acylcarnitines, non-esterified fatty acids (NEFA) and β -hydroxybutyrate (β -OH) were determined as described in the Experimental section. Values are means $\pm$ s.d. Numbers of animals used for determinations of [NEFA] and [β -OH] are shown in parentheses when different from the numbers for carnitine determinations.

Time (min)	n	[Carnitine] (μM)	[Acid-soluble acylcarnitines] (μM)	[Acid-soluble acylcarnitines]/	[NEFA] (mM)	[β -OH] (mM)
				[carnitine]		
0	14	15 $\pm$ 3	24 $\pm$ 5	1.71 $\pm$ 0.57	0.65 $\pm$ 0.24 (20)	1.88 $\pm$ 0.51
2	4	3050 $\pm$ 590	180 $\pm$ 120	0.06 $\pm$ 0.03	—	—
5	6	1620 $\pm$ 250	100 $\pm$ 70	0.06 $\pm$ 0.04	0.88 $\pm$ 0.19	1.55 $\pm$ 0.51
10	3	1550 $\pm$ 160	57 $\pm$ 60	0.04 $\pm$ 0.05	0.89 $\pm$ 0.41	1.52 $\pm$ 0.57
20	4	950 $\pm$ 350	190 $\pm$ 90	0.26 $\pm$ 0.20	0.81 $\pm$ 0.09	1.93 $\pm$ 0.20
30	3	380 $\pm$ 85	260 $\pm$ 10	0.70 $\pm$ 0.14	0.84 $\pm$ 0.25	2.38 $\pm$ 0.44
60	5	130 $\pm$ 50	220 $\pm$ 40	1.92 $\pm$ 0.62	0.54 $\pm$ 0.13	1.90 $\pm$ 0.56
120	7	53 $\pm$ 14	190 $\pm$ 20	2.64 $\pm$ 0.96	0.50 $\pm$ 0.17	1.48 $\pm$ 0.69

increased after carnitine administration. Total carnitine content reached a value five and a half times that of controls before decreasing. The liver was able to take up approx. 1 μmol of (—)-carnitine/g during the 10 min after injection.

The formation of acylcarnitines indicates that the carnitine taken up by the liver has access to the acyl-CoA pool through the carnitine acyltransferases. Further the liver has a source of acyl-CoA molecules that can be utilized for the production of acylcarnitines. As Table 3 indicates, the ratio of acid-soluble acylcarnitines to carnitine remained relatively constant. This suggests that the change in acid-soluble acylcarnitines was the result of mass action, with acylcarnitines increasing in response to the increased carnitine and maintaining the system at a steady state.

Data on the hepatic carnitine pool for 24-h-

starved animals after carnitine injection are presented in Table 4. While the pattern of changes was similar to that seen in fed rats, several important differences were noted. The peak liver total carnitine content in the starved animals was 2.33 $\mu\text{mol}/\text{g}$, more than one and a half times that seen in the fed rats. In contrast, the carnitine content was less than that seen in the fed state at time points after 10 min. This discrepancy was accounted for by the marked increase in acylcarnitines (both acid-soluble and acid-insoluble) seen in starved rats. Examination of the acid-soluble acylcarnitine/carnitine ratio for the starved rats (Table 4) reveals that, although at 2 min after injection the ratio was significantly decreased ($P < 0.01$), at subsequent time points it was increased ($P < 0.01$ at 20, 30 and 60 min compared with time zero). At 20 min after injection the ratio was almost double that from uninjected animals. The

Table 3. *Liver acid-soluble carnitines after carnitine administration to fed rats*

(—) Carnitine at a dose of 60 μ mol/100g body wt. was given to fed rats. At the times indicated animals were killed and liver carnitine, acid-soluble acylcarnitines, acid-insoluble acylcarnitines and total carnitine concentrations were determined as described in the Experimental section. Values are means $\pm$ S.D.

Time (min)	<i>n</i>	Carnitine (nmol/g)	Acid-soluble acylcarnitines (nmol/g)	[Acid-soluble acylcarnitines]/ [carnitine]	Acid-insoluble acylcarnitines (nmol/g)	Total carnitine (nmol/g)
0	16	130 $\pm$ 30	110 $\pm$ 40	0.90 $\pm$ 0.34	19 $\pm$ 7	260 $\pm$ 54
1	5	280 $\pm$ 70	190 $\pm$ 40	0.71 $\pm$ 0.29	36 $\pm$ 5	500 $\pm$ 74
5	7	370 $\pm$ 140	360 $\pm$ 80	1.08 $\pm$ 0.38	55 $\pm$ 19	790 $\pm$ 210
10	3	620 $\pm$ 15	600 $\pm$ 140	0.97 $\pm$ 0.26	79 $\pm$ 18	1300 $\pm$ 140
20	3	610 $\pm$ 220	510 $\pm$ 50	0.93 $\pm$ 0.37	55 $\pm$ 13	1200 $\pm$ 170
30	6	810 $\pm$ 340	590 $\pm$ 150	0.89 $\pm$ 0.50	56 $\pm$ 13	1500 $\pm$ 280
60	4	580 $\pm$ 140	620 $\pm$ 230	1.14 $\pm$ 0.58	47 $\pm$ 5	1200 $\pm$ 200
120	6	460 $\pm$ 130	460 $\pm$ 50	1.03 $\pm$ 0.23	46 $\pm$ 15	970 $\pm$ 190

Table 4. *Liver acid-soluble carnitines after carnitine administration to 24-h-starved rats*

(—) Carnitine at a dose of 60 μ mol/100g body wt. was given to 24-h-starved rats. At the times indicated animals were killed and liver carnitine, acid-soluble acylcarnitines, acid-insoluble acylcarnitines and total carnitine concentrations were determined as described in the Experimental section. Values are means $\pm$ S.D.

Time (min)	<i>n</i>	Carnitine (nmol/g)	Acid-soluble acylcarnitines (nmol/g)	[Acid-soluble acylcarnitines]/ [carnitine]	Acid-insoluble acylcarnitines (nmol/g)	Total carnitine (nmol/g)
0	14	190 $\pm$ 70	210 $\pm$ 50	1.27 $\pm$ 0.65	87 $\pm$ 19	480 $\pm$ 88
2	4	520 $\pm$ 160	250 $\pm$ 90	0.48 $\pm$ 0.14	110 $\pm$ 30	880 $\pm$ 260
5	6	440 $\pm$ 130	590 $\pm$ 120	1.40 $\pm$ 0.23	230 $\pm$ 71	1300 $\pm$ 210
10	3	440 $\pm$ 80	710 $\pm$ 80	1.63 $\pm$ 0.12	320 $\pm$ 360	1500 $\pm$ 10
20	4	600 $\pm$ 80	1450 $\pm$ 420	2.40 $\pm$ 0.45	290 $\pm$ 97	2330 $\pm$ 490
30	3	500 $\pm$ 100	1050 $\pm$ 340	2.07 $\pm$ 0.33	340 $\pm$ 160	1900 $\pm$ 320
60	5	460 $\pm$ 60	1060 $\pm$ 210	2.31 $\pm$ 0.46	160 $\pm$ 54	1700 $\pm$ 270
120	7	330 $\pm$ 50	490 $\pm$ 70	1.50 $\pm$ 0.21	110 $\pm$ 37	930 $\pm$ 100

mechanism by which carnitine administration caused an increase in the acylcarnitine/carnitine ratio in starved rats is unclear, but suggests that the starving animal possesses a larger accessible source of acyl-CoA molecules for acylcarnitine formation compared with fed rats.

Acetylcarnitine represents an important acid-soluble acylcarnitine because of its generation from, and conversion into, acetyl-CoA. For this reason acetylcarnitine was specifically measured in liver samples from the injected rats. Table 5 shows that hepatic acetylcarnitine content increased in both fed and starved rats after carnitine injection. When all acid-soluble acylcarnitines were measured (Table 3), a larger increase was observed in starved animals. This was also the case with acetylcarnitine, as twice as much acetylcarnitine was formed in starved compared with fed rats. Table 5 also shows the proportion of hepatic acid-soluble acylcarnitines that was acetylcarnitine at each time point. In fed control animals acetylcarnitine made up 37% of acid-soluble

acylcarnitines, and this proportion did not change significantly ($P > 0.05$) at any time after injection. Acid-soluble acylcarnitines in liver from starved animals were 53% acetylcarnitine and this percentage increased significantly ($P < 0.01$ at 2 and 5 min, $P < 0.05$ at 10 and 60 min when compared with time zero) after carnitine injection. This was particularly marked during the first 10 min after injection, when acetylcarnitine made up more than 70% of all acid-soluble acylcarnitines.

Despite this evidence that the exogenous carnitine was incorporated into the rat's metabolism, no major changes were observed in the plasma β -hydroxybutyrate or non-esterified fatty acid concentrations (Tables 1 and 2). In both fed and starved animals there was no significant change in β -hydroxybutyrate concentration during the 2 h after carnitine administration. Non-esterified fatty acid concentrations did not change in the fed state, and were only increased slightly in the starved animals (significantly increased at the 5 min time point

Table 5. *Liver acetylcarnitine after carnitine administration to fed and 24-h-starved rats*

(—) Carnitine at a dose of 60 µmol/100g body wt. was given to fed or 24-h-starved rats. At the times indicated animals were killed and liver acetylcarnitine was determined as described in the Experimental section. Values are means ± S.D.

Time (min)	Fed rat		Starved rat	
	Acetylcarnitine (nmol/g)	[Acetylcarnitine]/[acid-soluble acylcarnitine]	Acetylcarnitine (nmol/g)	[Acetylcarnitine]/[acid-soluble acylcarnitine]
0	40 ± 15	0.37 ± 0.13	110 ± 30	0.53 ± 0.13
1	70 ± 10	0.32 ± 0.08	—	—
2	—	—	200 ± 60	0.76 ± 0.21
5	150 ± 80	0.41 ± 0.17	440 ± 70	0.73 ± 0.09
10	280 ± 120	0.45 ± 0.10	530 ± 80	0.73 ± 0.10
20	150 ± 50	0.30 ± 0.13	890 ± 200	0.62 ± 0.07
30	230 ± 60	0.41 ± 0.18	650 ± 90	0.63 ± 0.12
60	210 ± 40	0.36 ± 0.11	710 ± 190	0.66 ± 0.16
120	150 ± 40	0.31 ± 0.08	330 ± 130	0.65 ± 0.20

Table 6. *Liver CoA and acyl-CoA contents after carnitine administration to fed rats*

(—) Carnitine at a dose of 60 µmol/100g body wt. was given to fed rats. Animals were killed at the times indicated, and liver acetyl-CoA, total acid-soluble CoA, the sum of acetyl-CoA and CoA, acid-insoluble CoA and total CoA were determined as described in the Experimental section. Values are means ± S.D.

Time (min)	Content (nmol/g wet wt. of liver)				
	Sum of acetyl-CoA and CoA	Acetyl-CoA	Total acid-soluble CoA	Acid-insoluble CoA	Total CoA
0	180 ± 50	70 ± 20	260 ± 50	88 ± 26	340 ± 60
1	180 ± 50	90 ± 10	210 ± 100	81 ± 14	310 ± 50
5	150 ± 30	66 ± 9	200 ± 80	120 ± 20	340 ± 60
10	170 ± 40	69 ± 11	210 ± 50	110 ± 4	320 ± 40
20	—	66 ± 11	—	—	—
30	150 ± 20	68 ± 15	230 ± 40	87 ± 19	320 ± 50
60	170 ± 20	68 ± 16	240 ± 60	88 ± 16	330 ± 80
120	140 ± 60	74 ± 11	190 ± 50	94 ± 24	290 ± 70

$P < 0.05$, otherwise $P > 0.05$). Tables 1 and 2 represent the means for several groups of animals, with control animals in each group. When the injected animals were compared with control animals from the same group the increase in non-esterified fatty acids observed in the starved animals was no longer significant.

The relationship between the hepatic carnitine and CoA pools was studied by measurement of acetyl-CoA, the sum of coenzyme A and acetyl-CoA, total acid-soluble CoA, acid-insoluble acyl-CoA and total CoA in the livers from carnitine-injected animals. Table 6 presents the hepatic CoA pool in fed animals after injection of (—) carnitine (60 µmol/100g body wt.). Despite the generation of large amounts of acetylcarnitine (Table 5), the only significant change

($P < 0.05$) in acetyl-CoA content was a small increase 1 min after injection. Total acid-soluble CoA decreased after injection, being significantly decreased ($P < 0.05$) 5 min after injection. The total acid-soluble CoA content then increased slowly toward control values. A significant decrease was also seen at 120 min. The acid-insoluble acyl-CoA content increased after carnitine administration and was significantly increased after 5 and 10 min. As expected, total hepatic CoA remained constant except after 120 min when it was decreased. This explains why total acid-soluble CoA was decreased at the 120 min time point. Since total CoA remained constant, the decrease in total acid-soluble CoA seen at 5 min can be accounted for by the increase in acid-insoluble acyl-CoA.

Table 7. *Liver CoA and acyl-CoA contents after carnitine administration to 24-h-starved rats*

(—) Carnitine at a dose of 60 $\mu\text{mol}/100\text{g}$ body wt. was given to 24-h-starved rats. Animals were killed at the times indicated, and liver acetyl-CoA, total acid-soluble CoA the sum of acetyl-CoA and CoA, acid-insoluble CoA and total CoA were determined as described in the Experimental section. Values are means $\pm$ S.D.

Content (nmol/g wet wt. of liver)

Time (min)	Sum of acetyl-CoA and CoA	Acetyl-CoA	Total acid-soluble CoA	Acid-insoluble CoA	Total CoA
0	180 $\pm$ 70	73 $\pm$ 24	240 $\pm$ 50	170 $\pm$ 40	420 $\pm$ 90
5	140 $\pm$ 70	83 $\pm$ 29	210 $\pm$ 40	190 $\pm$ 30	330 $\pm$ 150
10	150 $\pm$ 90	87 $\pm$ 6	240 $\pm$ 40	170 $\pm$ 50	410 $\pm$ 20
20	140 $\pm$ 70	88 $\pm$ 10	230 $\pm$ 40	160 $\pm$ 30	390 $\pm$ 10
30	130 $\pm$ 70	78 $\pm$ 16	200 $\pm$ 30	180 $\pm$ 30	380 $\pm$ 60
60	150 $\pm$ 70	78 $\pm$ 22	210 $\pm$ 30	130 $\pm$ 30	330 $\pm$ 50
120	150 $\pm$ 70	73 $\pm$ 18	220 $\pm$ 30	120 $\pm$ 30	340 $\pm$ 60

Similar data on the hepatic CoA pool after carnitine administration to 24-h-starved rats are shown in Table 7. As in the fed rats, no change was seen in the hepatic acetyl-CoA content after injection. Acid-insoluble acyl-CoA content remained constant for the first 30 min after injection, but was significantly ($P < 0.05$) decreased at 60 and 120 min. Because variations were also seen in total CoA content [decreased at 5, 60 ($P < 0.05$) and 120 min ($0.10 > P > 0.05$)] it is unclear whether these decreases in acid-insoluble acyl-CoA were owing to carnitine, reflect a smaller total CoA pool or reflect a decrease in the total CoA pool caused by carnitine. It seems unlikely that the decrease resulted from a direct action of carnitine since, as Table 4 shows, hepatic carnitine and acid-insoluble acylcarnitines are decreasing at these time points.

Discussion

The studies reported in the present paper demonstrate that carnitine administered by intravenous injection to fed and starved rats was integrated into the endogenous carnitine pool. This included the formation of large amounts of acylcarnitines. Differences existed in carnitine handling between starving and fed animals, but these differences reflected the endogenous carnitine pools in the two metabolic states. Despite these major changes in the animal's carnitine pool, only minimal changes were seen in other aspects of the animal's metabolism. The hepatic CoA pool changed only minimally after carnitine administration. Recent studies *in vitro* (Brass & Hoppel, 1980) have shown that under conditions maximal to both acyl-CoA and acetyl-carnitine production in rat liver mitochondria, only 5% of acetyl groups generated could be converted into acetylcarnitine. This is consistent with the resistance of the hepatic CoA pool to carnitine-

induced changes observed in the present paper *in vivo* and suggests this resistance is the result of a much higher flux of acyl groups through the CoA pool compared with the carnitine pool.

After carnitine administration the plasma compartment initially contains the entire dose. The carnitine is then cleared from the plasma by uptake into tissues and irreversible elimination (i.e. removed by the kidney to be excreted in the urine). There appear to be no differences between fed and starved rats in the removal of carnitine from the plasma at the dose given. A difference was observed in the uptake by liver of carnitine in the two metabolic states. On a per-gram basis the liver from starved rats appears to be more efficient in taking up carnitine than the liver from fed rats, but this may be secondary to the loss of liver mass during starvation. In this case the carnitine uptake per whole liver would be very similar. This possibility is supported by the similarity of the relative hepatic total carnitine uptake (approx. 1.8 $\mu\text{mol}/\text{g}$ vs. 1.2 $\mu\text{mol}/\text{g}$) and DNA content [approx. 5.5 mg/g vs. 3.4 mg/g (Herrera & Freinkel, 1968; Brass & Hoppel, 1978a)] in starved and fed animals respectively. Another possibility is that the higher total carnitine uptake in liver from starved rats was secondary to increased esterification to acylcarnitines, which served to 'pull' carnitine from the circulation.

The mechanism by which carnitine enters cells is not fully understood, but recent evidence has suggested an active-transport mechanism (Christiansen & Bremer, 1976; Molstad *et al.*, 1977). Because these studies were done *in vitro* their relation to handling of carnitine *in vivo* is unclear. In the studies reported in the present paper, liver was able to take up 1–2 μmol of carnitine/g in the first 10–20 min after carnitine administration. With isolated liver cells Christiansen & Bremer (1976) have

described an active-transport system for carnitine with a maximal velocity of approx. $0.5 \mu\text{mol}/\text{min}$ per g of liver, a rate high enough to allow for the uptake observed in the present study *in vivo*.

Because acid-soluble acylcarnitines are determined by the difference in total acid-soluble carnitine and carnitine, their concentrations cannot be determined accurately in the presence of extremely high carnitine concentrations. For this reason it is unclear whether the large acid-soluble acylcarnitine concentrations present at the earliest time points are artefacts of the measurement technique or represent a pool of rapidly appearing acylcarnitines, perhaps generated in the formed elements of the blood. Another possibility is that these acylcarnitines are endogenous and appear in the plasma as part of a carnitine-acylcarnitine-exchange tissue-uptake system. Although further studies are required to clarify these points, several important points can still be made from the plasma acid-soluble acylcarnitine concentrations as measured in the present paper. There was unquestionably a marked increase in the plasma carnitine/acylcarnitine ratio in the 30 min after injection and this ratio slowly returned toward the pre-injection value over the next 90 min. A definite peak in plasma acid-soluble acylcarnitines was observed during the first 30 min before the concentrations began to decrease along with the plasma carnitine concentration. These conclusions are not dependent on the possibly distorted acid-soluble acylcarnitine concentration seen at 1 and 5 min.

The large increases in hepatic acylcarnitine content seen in fed and 24-h-starved rats point out several interesting features of the carnitine-CoA interactions. The production of acylcarnitine after injection appeared to be characteristic of the metabolic state of the animal. In fed and 24-h-starved animals the acid-soluble acylcarnitine/carnitine ratio was different before injection, and this difference was maintained or accentuated after carnitine injection. This confirms the concept that the status of the hepatic carnitine pool reflects, and results from, the metabolic state of the liver, rather than changes in the carnitine pool causing changes in hepatic metabolism.

No changes were seen in the usual indices of fatty acid metabolism, plasma non-esterified fatty acids and ketone bodies, despite the interaction between the exogenous carnitine and the hepatic CoA pool. Further, only minimal changes were seen in the liver CoA pool itself. Although certain types of changes could not be detected in these studies, such as compensated for shifts in the acyl-CoA pool or re-distribution of acyl-CoA molecules within the cell, the lack of changes may reflect the relative fluxes in the carnitine and CoA pools as mentioned previously. The CoA pool is obviously very active with

all metabolized acyl groups passing through the pool resulting in a high flux. Therefore, the shunting of $1 \mu\text{mol}$ of acyl groups towards acylcarnitine production over 20 min will be of little significance compared with the estimated $30 \mu\text{mol}$ of C_2 units passing through the CoA pool during this period. [This calculation is based on 1 g of liver containing 60 mg of mitochondrial protein oxidizing 3 nmol of palmitate/min per mg of mitochondrial protein. This oxidation rate represents approx. 50% of capacity (DiMarco, 1973; DiMarco & Hoppel, 1975).] However, if the acylcarnitine pools turned over even a few times during the 20 min period, the amount of acyl groups required would be expected to cause a significant 'drain' on hepatic metabolism and the CoA pool. This provides a further indication that the acid-soluble acylcarnitine pool is metabolically sluggish, and supports the concept that the carnitine pool simply responds to changes in the CoA pool and the metabolic state of the animal.

The high values for hepatic acetyl-CoA content and the lack of significant change with starvation (time 0, Tables 6 and 7) reported in the present study were initially surprising. However, a review of the literature revealed several points. (1) There is great variation in reported concentrations and amount of change with starvation for rat liver acetyl-CoA, ranging from a 30% increase (Herrera & Freinkel, 1968) to the solitary report of a 4-fold increase by Guynn *et al.* (1972). (2) There is almost certainly a re-distribution of acetyl-CoA (see for example, Tischler *et al.*, 1977) within the cell, minimizing the usefulness of acetyl-CoA/g of liver as a measure of change in acetyl-CoA metabolism. (3) On a total-liver basis there is probably a decrease in acetyl-CoA with starvation, further confusing the issue as to availability or amount of acetyl-CoA being critical in the transition of metabolic state. Although these points do not explain our acetyl-CoA values, they present a perspective from which they should be examined. There is clearly a large variation in absolute acetyl-CoA concentrations in the literature, the origin of which is not clear. Our acetyl-CoA values have a larger intra-animal variation (S.D.) than those in the literature and this might have masked an increase in acetyl-CoA of the order of 30–40%, as reported by Herrera & Freinkel (1968).

It has been suggested previously (Brass & Hoppel, 1978b) that the increase in hepatic acid-insoluble acylcarnitine seen in the fed rats injected with carnitine without an increase in plasma β -hydroxybutyrate is inconsistent with carnitine palmitoyltransferase A activity being rate limiting and regulatory for ketogenesis. A similar observation is reported in the present paper in starved animals. It is recognized that ketone-body synthesis is not at capacity during starvation (DiMarco & Hoppel, 1975). Thus, the further increment in

acid-insoluble acylcarnitines seen after carnitine injection in the starved animals without a further increment in β -hydroxybutyrate concentration is also inconsistent with carnitine palmitoyltransferase A being rate limiting for ketogenesis. Several additional aspects of the present study support this hypothesis. In animals injected with carnitine no decrease in hepatic acetyl-CoA or acid-insoluble acyl-CoA was observed, ruling out a masking of increased ketone-body production by shunting of acyl groups away from acetoacetate production. An increase in acid-insoluble acyl-CoA content was seen in fed animals, suggesting that the increased acid-insoluble acylcarnitine content causes an increased acid-insoluble acyl-CoA production by carnitine palmitoyltransferase B. Since no anti-ketogenic effect of carnitine was seen in starved animals, no other mechanisms by which high carnitine concentrations can prevent ketone-body production are plausible.

Although the present studies clarify the nature of the interaction between carnitine and the hepatic CoA pool, several fundamental questions remain. The data discussed here are consistent with the concept that acylcarnitines serve to buffer or protect the hepatic CoA pool from large changes. Although this may explain the changes seen in acid-soluble acylcarnitines, it does not rule out a larger role for the acid-soluble acylcarnitines in metabolism. In addition to discussing the amount of acid-soluble acylcarnitines, identification of the specific acyl moieties is critical in evaluating changes in this group of compounds. Another important type of change in the liver CoA pool that could not be evaluated in the present study is a redistribution of acyl-CoA molecules between the cytoplasm and mitochondrial compartments. Such a shift would be expected as the hepatic metabolism shifts from utilization of acyl-CoA in the cytoplasm (acetyl-CoA for fatty acid synthesis and acid-insoluble acyl-CoA for esterification) to their oxidative metabolism in the mitochondria.

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