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# Urinary Excretion of *l*-Carnitine and Acylcarnitines by Patients with Disorders of Organic Acid Metabolism: Evidence for Secondary Insufficiency of *l*-Carnitine

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**ABSTRACT. Concentrations of *l*-carnitine and acylcarnitines have been determined in urine from patients with disorders of organic acid metabolism associated with an intramitochondrial accumulation of acyl-CoA intermediates. These included propionic acidemia, methylmalonic aciduria, isovaleric acidemia, multicarboxylase deficiency, 3-hydroxy-3-methylglutaric aciduria, methylacetoacetyl-CoA thiolase deficiency, and various dicarboxylic acidurias including glutaric aciduria, medium-chain acyl-CoA dehydrogenase deficiency, and multiple acyl-CoA dehydrogenase deficiency. In all cases, concentrations of acylcarnitines were greatly increased above normal with free carnitine concentrations ranging from undetectable to supranormal values. The ratios of acylcarnitine/carnitine were elevated above the normal value of 2.0 ± 1.1. *l*-Carnitine was given to three of these patients; in each case, concentrations of plasma and urine carnitines increased accompanied by a marked increase in concentrations of short-chain acylcarnitines. These acylcarnitines have been examined using fast atom bombardment mass spectrometry in some of these diseases and have been shown to be propionylcarnitine in methylmalonic aciduria and propionic acidemia, isovalerylcarnitine in isovaleric acidemia, and hexanoylcarnitine and octanoylcarnitine in medium-chain acyl-CoA dehydrogenase deficiency. The excretion of these acylcarnitines is compatible with the known accumulation of the corresponding acyl-CoA esters in these diseases.**

**In this group of disorders, the increased acylcarnitine/carnitine ratio in urine and plasma indicates an imbalance of mitochondrial mass action homeostasis and, hence, of acyl-CoA/CoA ratios. Despite naturally occurring attempts to increase endogeneous *l*-carnitine biosynthesis, there is insufficient carnitine available to restore the mass action ratio as demonstrated by the further increase in acylcarnitine excretion when patients were given oral *l*-carnitine. Thus, *l*-carnitine insufficiency is a general phenomenon in these diseases. (*Pediatr Res* 18:1325–1328, 1984)**

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Roe and Bohun (15) observed absence of free *l*-carnitine in urine of a patient with propionic acidemia (propionyl-CoA carboxylase deficiency), with favorable clinical responses to *l*-carnitine challenge and treatment. This observation prompted reports of reduced plasma free carnitine in patients with a variety of other metabolic disorders (1, 16) and Seccombe *et al.* (18) also reported increased acylcarnitine to carnitine ratios in a patient with methylmalonic aciduria. Similar observations of reduced plasma carnitine and increased acylcarnitine excretion in a patient with multiple acyl-CoA dehydrogenation defects ("glutaric aciduria type II") have been made (11). The importance of measurement of acylcarnitine concentrations in such conditions was underlined by the observation that under ketotic conditions acylcarnitine concentrations increase at the expense of free carnitine (12). This suggests that a shift in mitochondrial metabolite ratios and mass action homeostasis occurs under these circumstances. We have recently studied a patient with methylmalonic aciduria with detailed observations of organic acid, carnitine, and acylcarnitine concentrations in plasma and urine before and during challenge with *l*-carnitine (16). Our results have emphasized the importance of measuring acylcarnitines as well as free carnitine and the value of urinary concentrations in assessing the carnitine status and dynamics in such patients. These observations and those of others (11) suggest secondary carnitine insufficiency may be a general phenomenon in disorders of organic acid metabolism in which acyl-CoA intermediates accumulate. The present paper reports the results of studies made on urine specimens obtained from 35 patients with such disorders that, together with plasma and urine data during carnitine challenges on selected patients, support this concept. Some of these results have been reported briefly in abstract form elsewhere (6, 7).

## PATIENTS AND METHODS

Urine specimens were obtained from patients with disorders of branched-chain amino acid metabolism, with disorders of L-lysine metabolism, and with various nonketotic dicarboxylic acidurias in which the abnormal organic aciduria is consistent with a reduced capacity for β-oxidation. They included seven patients with methylmalonic aciduria including one $B_{12}$-responsive patient and one patient with combined methylmalonic aciduria and homocystinuria, two patients with isovaleric acidemia (isovaleryl-CoA dehydrogenase deficiency), two patients with multicarboxylase deficiency (combined deficiencies of pro-

pionyl-CoA carboxylase, 3-methylcrotonyl-CoA carboxylase, and pyruvate carboxylase), three patients with 3-hydroxy-3-methylglutaric aciduria (3-hydroxy-3-methylglutaryl-CoA lyase deficiency) and two patients with 2-methyl-3-hydroxybutyric aciduria and tiglylglycinuria (β-ketothiolase or methylacetoacetyl-CoA thiolase deficiency). The patients with dicarboxylic aciduria included two with glutaric aciduria ("type I"; glutaryl-CoA dehydrogenase deficiency), and one with multiple acyl-CoA dehydrogenase deficiency ("glutaric aciduria type II"). The five other patients with nonketotic dicarboxylic aciduria included three with medium-chain acyl-CoA dehydrogenase deficiency associated with suberylglycinuria and two others with less well defined disorders. The clinical and biochemical features of all these disorders have been reviewed elsewhere (5).

All of these patients have been previously diagnosed on the basis of their clinical presentation, abnormal organic aciduria as determined using gas chromatography and mass spectrometry, and demonstration of deficient or abnormal enzyme activity in cultured skin fibroblasts whenever possible. In most cases, the diagnosis had been confirmed by biochemical studies in two centers. Ages of the patients ranged between 3 months and 12 years.

Plasma and urine *l*-carnitine and acylcarnitines were determined using radioenzymatic assays utilizing [1-$^{14}$C]acetyl-CoA and carnitine acetyltransferase (3). The concentrations in plasma are expressed in μmol/liter for free carnitine, short-chain acid-soluble acylcarnitines, and long-chain acid-insoluble acylcarnitines. Those in urine are expressed in nmol/mg creatinine for free carnitine and acylcarnitines. Normal values for carnitine and acylcarnitines in plasma and urine from an adult control population ($n$ = 10) were used for the present study. Values obtained are comparable with those reported elsewhere and to preliminary data obtained in children and adults in the age range of 3 weeks to 52 years ($n$ = 58) (C. de Sousa, R. A. Chalmers, and T. E. Stacey, unpublished data). Urinary creatinine concentrations were determined using an established alkaline picrate method.

Acylcarnitines were identified in urine samples using fast atom bombardment mass spectrometry with high resolution mass measurements and linked scanning techniques as described elsewhere (16, 17).

## RESULTS

Concentrations of carnitine and acylcarnitines in urine of patients with propionic acidemia and methylmalonic aciduria are shown in Table 1. While concentrations of free carnitine range from undetectable to supranormal values, in all cases, the concentrations of acylcarnitines are greatly increased. In particular, the ratio of acylcarnitine/carnitine is elevated above the normal value of 2.0 ± 1.1. Three of these patients (patients 3 and 4 with propionic acidemia and patient 12 with vitamin $B_{12}$-unresponsive methylmalonic aciduria) have received *l*-carnitine in single oral doses of 25 (patients 3 and 4) and 100 mg/kg body weight (patient 12). In each case, there was an increase in plasma concentration of free carnitine accompanied by a marked increase in concentrations of short-chain acylcarnitines (Table 2). Although increases were also observed in plasma long-chain acylcarnitines, these never achieved values within the normal range. Even more marked changes were observed in urine, with very large increases in the concentration of acylcarnitines (Table 2).

Table 3 shows the concentrations of free carnitine and acylcarnitines in urine of patients with other disorders of branched-chain amino acid metabolism. In all cases, the acylcarnitine excretion and the acylcarnitine/carnitine ratios are greatly increased above the normal values.

Table 4 shows the results obtained in patients with glutaric aciduria and with other metabolic disorders associated with increased excretion of dicarboxylic acids. Again, in all patients, the excretion of acylcarnitines is greatly increased above normal with an increased acylcarnitine/carnitine ratio.

The major acylcarnitine excreted by patients with methylmalonic aciduria and with propionic acidemia has been shown to be propionylcarnitine. Acylcarnitines excreted by patients with isovaleric acidemia and with medium chain acyl-CoA dehydrogenase deficiency have been identified as isovalerylcarnitine and as hexanoylcarnitine and octanoylcarnitine, respectively.

## DISCUSSION

*l*-Carnitine (3-hydroxy-4-*N*-trimethylammoniobutanoate) is derived in man both from diet and from endogenous biosynthesis

Table 1. *Urinary carnitine distribution in patients with propionic acidemia and methylmalonic aciduria*

| Disorder | Patient No. | Carnitine (nmol/mg creatinine) Total | Free | Acylcarnitines | Acyl/Free |
|---|---|---|---|---|---|
| Propionic acidemia | 1 | 793 | 87 | 706 | 8.1 |
| | 2 | 911 | 104 | 808 | 7.8 |
| | 3 | 185 | 6 | 179 | 29.8 |
| | 4 | 327 | 48 | 279 | 5.8 |
| | 5 | 294 | 11 | 284 | 25.8 |
| | 6 | 241 | 4 | 237 | 59.3 |
| | 7 | 185 | <0.1 | 185 | >185 |
| Average | | 419 | 37 | 382 | 46 |
| Methylmalonic aciduria | 8 | 1015 | 98 | 917 | 9.4 |
| | 9 | 228 | 11 | 216 | 19.6 |
| | 10 | 1123 | 65 | 1058 | 16.3 |
| | 11 | 325 | 16 | 309 | 19.3 |
| | 12 | 494 | 142 | 352 | 2.5 |
| | 13 | 1081 | 19 | 912 | 5.4 |
| | 14 | 117 | <0.1 | 117 | >117 |
| | 15 | 517 | 102 | 415 | 4.1 |
| | 16 | 201 | 10 | 191 | 19.1 |
| | 17 | 73 | 0.5 | 73.5 | 147 |
| Average | | 517 | 61 | 456 | 36 |
| Normal values (±SD) | | 125 ± 75 | 51 ± 40 | 74 ± 40 | 2.0 ± 1.1 |
| Range | | 36–255 | 7–128 | 24–127 | 0.7–3.4 |

Table 2. *Effects of carnitine administration on plasma and urinary carnitine**

| Disorder | Patient No. | Carnitine (μmol/liter) Total | Free | Acid soluble (short-chain) | Acid insoluble (long-chain) |
|---|---|---|---|---|---|
| Plasma | | | | | |
| Propionic acidemia | 3 Pre | 30.1 | 4.6 | 24.5 | 1.0 |
| | Post | 160.2 | 47.4 | 57.1 | 1.7 |
| | 4 Pre | 37.5 | 11.7 | 24.6 | 1.2 |
| | Post | 97.8 | 41.4 | 54.2 | 2.2 |
| Methylmalonic aciduria | 12 Pre | 53.2 | 35.8 | 17.1 | 0.3 |
| | Post | 94.2 | 66.1 | 27.4 | 0.7 |
| Normal values (±SD) | | 46.1 ± 10.0 | 36.7 ± 7.6 | 5.7 ± 3.5 | 3.7 ± 1.5 |
| | | Carnitine (nmol/mg creatinine) | | | |
| | | Total | Free | Acyl | Acyl/free |
| Urine | | | | | |
| Propionic acidemia | 3 Pre | 185 | 6 | 179 | 29.8 |
| | Post | 4,916 | 1,429 | 3,487 | 2.4 |
| | 4 Pre | 327 | 48 | 279 | 5.8 |
| | Post | 4,806 | 738 | 4,068 | 5.5 |
| Methylmalonic aciduria | 12 Pre | 494 | 142 | 352 | 2.5 |
| | Post | 20,693 | 12,228 | 8,465 | 0.7 |
| Normal values (±SD) | | 125 ± 75 | 51 ± 40 | 74 ± 40 | 2.0 ± 1.1 |
| Range | | 36–255 | 7–128 | 24–127 | 0.7–3.4 |

* Carnitine was administered as a single oral dose of 25 mg/kg body weight to patients 3 and 4 and of 100 mg/kg body weight to patient 12; postcarnitine values refer to specimens collected 3 h after the dose was administered. Urine collected between +1 and +3 h.

Table 3. *Urinary carnitine distribution in patients with disorders of branched-chain amino acid metabolism**

| Disorder | Patient No. | Carnitine (nmol/mg creatinine) Total | Free | Acyl | Acyl/free |
|---|---|---|---|---|---|
| Isovaleric acidemia | 18 | 624 | 90 | 534 | 5.9 |
| | 19 | 243 | 26 | 217 | 8.4 |
| Multicarboxylase deficiency | 20 | 1008 | 69 | 340 | 13.6 |
| | 21 | 803 | 50 | 753 | 15.1 |
| | 22 | 433 | 15 | 417 | 27.8 |
| 3-Hydroxy-3-methylglutaric aciduria | 23 | 680 | 31 | 649 | 20.9 |
| | 24 | 98 | 6 | 92 | 15.3 |
| Methylacetoacetyl-CoA thiolase deficiency | 25 | 321 | 26 | 295 | 11.4 |
| | 26 | 316 | 6 | 309 | 51.5 |
| Normal values (±SD) | | 125 ± 75 | 51 ± 40 | 74 ± 40 | 2.0 ± 1.1 |
| Range | | 36–255 | 7–128 | 24–127 | 0.7–3.4 |

* Common names only are given for these disorders: fuller details are given in the text.

Table 4. *Urinary carnitine distribution in patients with disorders of organic acid metabolism associated with acyl-CoA accumulation and dicarboxylic aciduria*

| Disorder | Patient No. | Carnitine (nmol/mg creatinine) Total | Free | Acyl | Acyl/free |
|---|---|---|---|---|---|
| Glutaric aciduria | 27 | 460 | 4 | 456 | 114 |
| | 28 | 330 | 2 | 328 | 164 |
| Multiple acyl-CoA dehydrogenase deficiency | 29 | 633 | 43 | 590 | 13.7 |
| Suberylglycinuria (medium-chain acyl-CoA dehydrogenase deficiency) | 30 | 480 | 36 | 444 | 12.3 |
| | 31 | 307 | 141 | 166 | 1.2 |
| | 32 | 60 | 4 | 56 | 14.0 |
| Other dicarboxylic aciduria | 33 | 579 | 16 | 563 | 36.3 |
| | 34 | 210 | 16 | 194 | 12.1 |
| | 35 | 214 | 9 | 205 | 22.8 |
| Normal values (±SD) | | 125 ± 75 | 51 ± 40 | 74 ± 40 | 2.0 ± 1.1 |
| Range | | 36–255 | 7–128 | 24–127 | 0.7–3.4 |

(14). It has a classical role in the facilitation and modulation of transport of long-chain fatty acyl moieties across the inner mitochondrial membrane and, hence, of mitochondrial $\beta$-oxidation (6). Additional newly proposed roles for carnitine recognize the ability of carnitine to act as a cofactor in the transfer of acyl groups *out* of the mitochondrion, in the reverse direction to the classical role. In particular, the acetyl moiety of acetyl-CoA produced within the mitochondria may be transferred in this manner under certain conditions to the cytoplasm. It has been proposed that, by these means, the ratios of the concentrations of acyl-CoA to free CoA within the mitochondrion may be modulated (2, 6), which would in turn modulate the activity of key mitochondrial processes. Carnitine acyltransferases are steady state enzymes (13) with a random order mechanism (9, 10) relatively independent of pH changes within the physiological range (8). They follow the mass action route expressed in the equation: $([\text{acylcarnitine}]\cdot[\text{CoA}])/([\text{acyl-CoA}]\cdot[\text{carnitine}]) = K_{eq}$. Thus, a change in the ratio [acylcarnitine]/[carnitine] would reflect an opposite change in the ratio [acyl-CoA]/[CoA], and hence reflect the metabolic state of the individual (4). In any situation where acyl-CoA intermediates are accumulating within the mitochondria, the ratio [acyl-CoA]/[CoA] will increase dramatically, not only because of the increase in concentration of acyl-CoA but also because this increase will in itself result in a decrease in concentration of free CoA, the pool of total intramitochondrial CoA being finite in size. The effect of carnitine would then be to promote the formation of acylcarnitine according to the equilibrium reaction, resulting in reduction of [acyl-CoA] and increasing [CoA]. Unlike the acyl-CoA compounds however, the newly formed acylcarnitines are able to leave the mitochondria by means of the translocases, thereby further driving the reaction in the direction of reduction of [acyl-CoA] and production of free CoA. As a consequence the [acyl-CoA]/[CoA] ratio will be restored towards the normal mass action value, thereby increasing the availability of free CoA for its other key metabolic roles.

The data in this paper provide direct evidence for these concepts. In the patients studied, all of whom have enzyme deficiencies resulting in potential accumulation of intramitochondrial acyl-CoA compounds, there was an increase in excretion of acylcarnitines irrespective of the exact disorder. The precise identities of the acylcarnitines associated with all of the specific diseases remain to be established, but our studies using fast atom bombardment mass spectrometry have shown that the excreted acylcarnitines are compatible with the known intramitochondrial accumulation of their acyl-CoA precursors in the diseases concerned. Thus, propionylcarnitine is the major acylcarnitine excreted by patients with methylmalonic aciduria and propionic acidemia in which propionyl-CoA accumulates, isovalerylcarnitine is excreted by patients with isovaleric acidemia associated with isovaleryl-CoA accumulation, and hexanoylcarnitine and octanoylcarnitine by patients with medium chain acyl-CoA dehydrogenase deficiency. The major acylcarnitine excreted by normal subjects and patients with ketosis is acetylcarnitine.

In addition to the increased concentration of acylcarnitines in the urine of patients with these disorders, there is invariably an increase (often very great) in the ratio of acylcarnitine to free carnitine. This phenomenon is also seen in plasma. We interpret this as reflecting a pathological ratio of acyl-CoA to free CoA by the mass action phenomenon. An interesting consequence of this, of potential therapeutic importance, is the possibility that such patients may have insufficient carnitine for these excretory mechanisms to operate to maximum effect. This is paradoxical in that their excretion of total carnitine derivatives may be greatly increased, presumably as a result of supranormal synthesis since dietary sources are relatively normal or even reduced; such patients could scarcely be termed carnitine-deficient. Nevertheless, the concentrations of free carnitine are extremely low in some patients, suggesting that they are synthesizing insufficient carnitine for their increased metabolic needs. Further support for this concept of carnitine insufficiency is proved by the biochemical response shown in some of these patients to an additional oral load of exogenous *l*-carnitine which results in an even greater excretion of acylcarnitines (7, 16).

Thus, in this group of disorders, the increased acylcarnitine/carnitine ratio in urine and plasma supports the concept of an imbalance of mitochondrial mass action homeostasis. It is important to note that the acyl-CoA compounds such as propionyl-CoA that accumulate in these conditions are potentially extremely toxic due to their widespread effects on other metabolic pathways (16). There appears to be an attempt to compensate for this by increased production of carnitine, resulting in excretion (and hence detoxification at the mitochondrial level) of potentially toxic acyl groups as acylcarnitines. This increased production under basal conditions is apparently still not sufficient to restore the normal mass action ratio for there is further elimination of such acyl groups when patients are given oral *l*-carnitine. We believe *l*-carnitine supplementation may have a key role in the general management both acute and longer term of patients with disorders of organic acid metabolism, and our data confirm the necessity to consider acylcarnitine concentrations in addition to that of free carnitine when assessing the metabolic status of these patients.

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