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Carnitine deficiency in children and adolescents with type 1 diabetes

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Abstract

Carnitine is essential for the lipid and carbohydrate metabolism, and proper metabolic control in type 1 diabetes has potential impact on long-term complications. The plasma total, free, and acylcarnitine levels in 47 children and adolescents with type 1 diabetes were determined by a radioisotopic assay and compared to the values of a series of anthropometric measurements and metabolic parameters, including blood glycosylated hemoglobin Alc, serum cholesterol and triglycerides, and urine microalbumin levels. Plasma values for total, free, and acylcarnitine were 30.1±7.26, 20.0±4.50, and 10.2±6.47 μmol/l, respectively. Acyl/free carnitine ratio was 0.544±0.369. Individuals with type 1 diabetes had significantly lower total and free carnitine levels and significantly higher acyl/free carnitine ratios than controls ($P < .001$). Plasma total and free carnitine levels were inversely correlated to the duration of diabetes ($P = .036$ and $P = .071$, respectively). No statistical relationship was documented between carnitine levels and the remaining anthropometric and metabolic variables. In conclusion, total and free carnitine levels are decreased in children and adolescents with type 1 diabetes. This reduction is time related and may have potential interactions with the long-term complications of type 1 diabetes. Larger studies are required for final conclusions to be drawn on the precise role of carnitine and the possible benefit, if any, of carnitine supplementation in diabetic patients.

Keywords: Adolescence; Carnitine; Children; Diabetic complications; Type 1 diabetes

The importance of carnitine (3-hydroxy-4-*N*-trimethyl-aminobutyrate) for the lipid and carbohydrate metabolism has long been established (Proulx et al., 1997; Tamamogullari, Silig, Icagasioglu, & Atalay, 1999). Carnitine is required to transport long-chain fatty acids from the cytoplasm to the mitochondrial matrix where their β-oxidation occurs, and on the other hand, carnitine increases the sensitivity of the cells to insulin and the use of glucose by the peripheral tissues (Proulx et al., 1997; Tamamogullari et al., 1999).

In diabetes, metabolism of carbohydrate and fat is abnormal and an association between the proper metabolic control and the development of complications is widely recognized (Tamamogullari et al., 1999). Decreased plasma carnitine levels have already been reported in patients with type 2 diabetes (Okuda, Kawai, Murayama, & Yamashita, 1987; Pregant, Kaiser, & Schernthaner, 1993; Tamamogullari et al., 1999) and an underestimated role of carnitine in the clinical course and the complications has been suggested (Tamamogullari et al., 1999). Studies investigating the carnitine status in type 1 diabetes are extremely rare (Pregant et al., 1991; Soltesz, Melegh, & Sandor, 1983; Winter et al., 1989). The development of microvascular complications is uncommon during childhood and adolescence; however, the principles of proper metabolic control must be imparted from the onset. Recognition of carnitine deficiency in these patients may thus have therapeutic implications. In this study, we investigated the carnitine levels in children and adolescents with type 1 diabetes.

1. Subjects and methods

1.1. Subjects

Forty-seven children and adolescents with type 1 diabetes, all of them on insulin treatment and followed-up on an outpatient basis, were included in the study. Patients with chronic renal failure, liver cirrhosis, or on antiepileptic drugs were not included in the study as these conditions

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are associated with secondary carnitine deficiency (Jallon & Picard, 2001; Proulx et al., 1997; Rebouche & Paulson, 1986). Following informed consent from patients and their parents, plasma concentrations of the total, the free, and the acylcarnitine in fasting blood samples were measured and compared with the values of 30 healthy children and adolescents of similar gender and age who served as controls. Anthropometric parameters, including body weight and height, weight/height ratio, waist circumference/body height, and body mass index, were recorded and *z* scores (standard deviation scores) were calculated based on the Greek growth data (Mantziagrioti-Meimarides, Pantazides, Doxiadis, & Raphael, 1986). Blood glycosylated hemoglobin Alc levels as an indicator of metabolic control and urine microalbumin levels as an early indicator of diabetic nephropathy were determined. All the patients were screened for serum total cholesterol and triglycerides after a 12-h fast. All the aforementioned investigation was performed during the programmed 6-month follow-up of patients.

1.2. Methods

Venous blood samples were obtained, centrifuged, and kept frozen at −70 °C until analysis. Plasma total and free carnitine concentrations were measured by the radioisotopic method of McGarry and Foster (Bohles, Evangeliou, Bervoets, Eckert, & Sewell, 1994; Malone, Schocken, Morrison, & Gilbert-Barness, 1999; McGarry & Foster, 1976; Proulx et al., 1997; Schmidt-Sommerfeld, Werner, & Penn, 1988). The fraction of acylcarnitine was determined by subtracting free carnitine from the amount of total carnitine, which was determined after alkaline hydrolysis of all carnitine esters. The normal values for carnitine concentrations proposed by Schmidt-Sommerfeld et al. were used (Nicholson & Pesce, 2000; Schmidt-Sommerfeld et al., 1988; Soltesz et al., 1983). Low plasma carnitine was defined as a value <−2 S.D. Children were considered to be deficient of free carnitine when presenting with levels less than 20 μmol/l, and acylcarnitine levels were considered elevated when greater than 11 μmol/l (Winter et al., 1989). Glycosylated hemoglobin Alc levels were determined in capillary blood by Bayer DCA 2000 Analyser and serum total cholesterol and triglyceride levels by commercial kits. Twenty-four-hour urine specimens were collected and urine microalbumin levels were determined by immunochemistry (nephelometry, third generation Behring Nephelometer II).

Table 1
Clinical and metabolic parameters in 47 children and adolescents with type 1 diabetes

Parameter	Mean±S.D.	Range
Age at diagnosis (years)	8.10±4.08	0.2–17.3
Age at study (years)	13.2±4.35	4.2–19.6
Diabetes duration (years)	5.17±3.98	0.3–16.2
Body weight (*z* score)	0.556±1.04	−0.765–2.85
Body height (*z* score)	0.229±1.18	−1.55–2.70
Body weight/height (*z* score)	0.615±1.09	−0.930–2.92
Waist circumference/body height	0.474±0.047	0.384–0.594
Body mass index (kg/m^2)	20.8±3.84	14.5–29.6
Blood glycosylated hemoglobin (%)	8.25±1.67	4.90–13.6
Serum total cholesterol (mg/dl)	177±33.5	136–277
Serum triglycerides (mg/dl)	61.3±21.2	25–103
Urine microalbumin (mg/dl)	6.60±6.73	1.40–28.3

Table 2
Serum carnitine levels in 47 children and adolescents with type 1 diabetes and in 30 controls

Carnitine	Type 1 diabetes	Control group	*P*
Total carnitine (μmol/l)	30.1±7.26	46.1±6.42	<.001
Free carnitine (μmol/l)	20.0±4.50	37.9±5.04	<.001
Acylcarnitine (μmol/l)	10.2±6.47	8.17±2.35	.109
Acyl/free carnitine ratio	0.544±0.369	0.215±0.057	<.001

1.3. Statistics

The results are expressed as means±S.D. The Student *t* test, the analysis of variance, and the Pearson correlation test were used to determine the significance of difference and relationship. The conventional level of $P<.05$ was taken as the level of significance.

2. Results

Among the 47 children and adolescents with type 1 diabetes, 23 were males and 24 were females. Twenty-seven were residents of rural and semiurban and 20 of urban areas. Clinical, anthropometric, and metabolic characteristics are depicted in Table 1. All urinary microalbumin concentrations were normal. The total, free, and acylcarnitine plasma levels and the acyl/free carnitine ratio in both individuals with type 1 diabetes and in controls are depicted in Table 2. Children and adolescents with type 1 diabetes had significantly lower total and free carnitine levels and significantly higher acyl/free carnitine ratio than controls ($P<.001$).

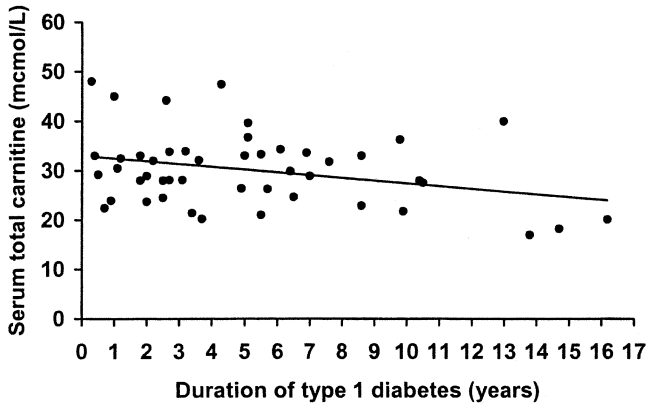

Fig. 1. Correlation between serum total carnitine and the duration of type 1 diabetes in 47 children and adolescents.

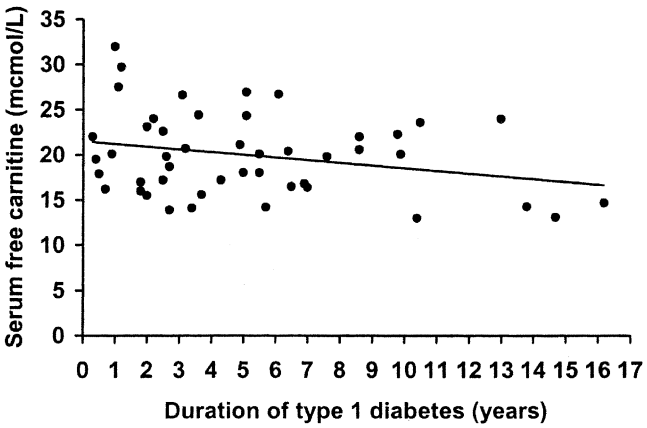

Fig. 2. Correlation between serum free carnitine and the duration of type 1 diabetes in 47 children and adolescents.

Acylcarnitine levels and age did not differ significantly between the two groups ($P=.109$ and .174, respectively). Total carnitine and free carnitine levels were below the normal range established for age in 36 and 31 patients (77% and 66%, respectively). Deficient of free carnitine were 24 patients (51%), and elevated acylcarnitine levels were found in 22 (47%).

Total, free, and acylcarnitine and acyl/free carnitine ratio values were not found related to gender, residence, age at diabetes onset, body weight, body height, body weight/height ratio, waist circumference/body height ratio, body mass index, blood glycosylated hemoglobin Alc, serum total cholesterol and triglycerides, and urine microalbumin levels. Total and free carnitine values were decreased as the duration of diabetes increased (Figs. 1 and 2; $r=-.304$ and $-.263$; $P=.036$ and .071, respectively). By contrast, acyl carnitine levels and acyl/free carnitine ratio were not significantly related to the duration of diabetes.

3. Discussion

Carnitine is the obligatory carrier of long- and medium-chain fatty acids from the cytoplasma into muscle mitochondria and increases fatty acid oxidation by activating the enzyme carnitine palmitoyl transferase in the outer membrane of the mitochondria (Athanassakis et al., 2001; Giannacopoulou et al., 1998; Pregant et al., 1993; Proulx et al., 1997; Rebouche & Paulson, 1986). Carnitine additionally regulates the intramitochondrial acyl to free coenzyme A ratio (Proulx et al., 1997; Rebouche & Paulson, 1986). Carnitine insufficiency, characterized by low free carnitine levels associated with an increased acyl/free carnitine ratio, may permit the accumulation of toxic products (Proulx et al., 1997). Additionally, carnitine is involved in the carbohydrate metabolism (Tamamogullari et al., 1999) and in the maintenance of cell membrane structure and cell viability and has been reported to reduce the apoptotic levels of CD4+ and CD8+ cells (Athanassakis et al., 2001). Although about 98% of total body carnitine is stored within skeletal muscle, decreased plasma carnitine levels have been related to low tissue concentrations (Proulx et al., 1997; Rebouche & Paulson, 1986).

Decreased plasma carnitine levels have already been reported in type 2 diabetes (Okuda et al., 1987; Pregant et al., 1993; Tamamogullari et al., 1999). Patients with retinopathy, hyperlipidemia, or neuropathy presented with low plasma total and free carnitine levels as compared to diabetic patients without complications (Tamamogullari et al., 1999). This finding has been confirmed in animal studies, and decreased plasma carnitine levels have been found in diabetic rats with neuropathy (Tamamogullari et al., 1999). Furthermore, a significant role of carnitine in the pathogenesis of diabetic cardiomyopathy has been suggested (Malone et al., 1999; Pregant et al., 1991). Chemically induced carnitine deficiency in nonhyperglycemic rats has resulted in increased serum free fatty acids, reduced glucose oxidation, and reduced cardiac function, which was acutely corrected by the addition of L-carnitine to the perfusion media bathing the explanted heart system (Malone et al., 1999).

Studies investigating the role of carnitine in type 1 diabetes are extremely rare (Pregant et al., 1991; Soltesz et al., 1983; Winter et al., 1989). These studies have shown decreased plasma levels of carnitine as compared to controls, and a potential role of this insufficiency in the pathogenesis of diabetes associated complications has been suggested. In our study this decrease in carnitine levels seems to be secondary to the reduction of free carnitine levels as acylcarnitine values were rather increased. As in inborn errors of metabolism, elevated plasma values of acylcarnitine suggest an increased production of CoA esters of organic acids with an increased mitochondrial workload. Similar findings demonstrating reduced free carnitine, elevated acylcarnitine, and an elevated acyl/free carnitine ratio have already been reported (Soltesz et al., 1983; Winter et al., 1989). Furthermore, these alterations have been demonstrated both in ketotic and ketoacidotic children (Soltesz et al., 1983). The increased acylation seems only partly responsible for the reduction of free carnitine in diabetic ketosis as no correlation has been documented between free carnitine and acylcarnitine (Soltesz et al., 1983). This lack of relationship was confirmed in the present study as well. Interestingly, no correlation has been observed between carnitine levels and glycosylated hemoglobin HbAlc (Pregant et al., 1991), and this was confirmed by the findings of the present study. The relationship of carnitine levels to the duration of type 1 diabetes has not been investigated up to date; our data suggest that free and total carnitine levels are reduced as the duration of diabetes increases.

The precise pathogenesis of carnitine deficiency in diabetic patients remains to be explored. Carnitine is both acquired from dietary sources and synthesized in the liver and kidneys from lysine and methionine. The major source

is the diet; however, the diet of the diabetic patients contains as much carnitine as of the general population. In animal models, carnitine deficiency has been attributed to reduced gastrointestinal absorption (Malone et al., 1999). Plasma carnitine concentrations are further subject to regulation by the kidneys (Huth & Shug, 1980) and urinary loss, plus the greater volume of urine from hyperglycemic animals has been suggested as the cause of the low plasma levels (Malone et al., 1999). However, studies investigating the free and acylcarnitine levels in urine have not revealed any relationship to the plasma levels (Proulx et al., 1997). The association of carnitine levels and renal function in diabetic patients has not been investigated up to date. Our findings do not indicate a relationship among the plasma total, free, and acylcarnitine levels and urine microalbumin levels, an early indicator of diabetic nephropathy.

Carnitine deficiency may have been an overlooked component in the management of diabetes. Fat oxidation with carnitine palmitoyl transferase inhibitors has been suggested as a promising emerging target (Wagman & Nuss, 2001), and nutritional strategies disinhibiting hepatic fatty acid oxidation (involving hydroxycitrate, carnitine, and pyruvate) have been suggested as well as potentially beneficial for type 2 diabetes (McCarty, 2000). Carnitine supplementation might thus be a simple means for the prevention of diabetic complications.

In conclusion, our data confirm the relative deficiency of total and free carnitine in children and adolescents with type 1 diabetes. Furthermore, clear evidence is provided that this decrease is time related and that patients with long-standing type 1 diabetes are prone to carnitine alterations. Larger studies are needed in order to draw firm conclusions and to explore a possible role, if any, of supplementary carnitine in the prevention of diabetic complications.

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