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SERUM AND URINE TOTAL, FREE AND ACYLCARNITINE LEVELS RELATED TO AGE: ASSESSMENT OF RENAL HANDLING OF CARNITINE*

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SUMMARY: Güneral F. (Central Laboratory, Department of Clinical Chemistry, University of Lausanne, Lausanne, Switzerland, and the Nutrition-Metabolism Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Serum and urine total, free and acylcarnitine levels related to age: assessment of renal handling of carnitine. 1995; 37: 217-222.

Control ranges of free, total and esterified carnitine in serum and spot urine samples of a healthy population of children and adults were established by radio-enzymatic assay. Children were divided into three groups according to age: six months to two years (n = 15), three to five years (n = 19), and six to ten years (n = 16). The adult group ranged in age from 20 to 35 years (n = 18). Serum free and total carnitine displayed a significant increase up to ten years of age, while acylcarnitines and the acylcarnitine/free carnitine ratio decreased until five years of age. Urinary free carnitine increased significantly up to five years of age. The overall increase in urinary total carnitine was insignificant. Urinary acylcarnitines and the acylcarnitine/free carnitine ratio decreased significantly in the three- to five-years age- group, increased significantly in the six- to ten-years age-group and remained constant afterwards. Renal handling of carnitine was assessed by evaluation of the renal reabsorption rate for free carnitine (RRFC) and the acylcarnitine/free carnitine clearance ratio (RAFCC), both of which were found to increase significantly up to five years of age. These data suggest that a balance in carnitine metabolism and excretion seems to be reached only after five years of age. The establishment of reference ranges in different population groups is critical for the investigation of children suspected to suffer from inherited metabolic disorders. *Key words: free carnitine, acylcarnitines, children, carnitine reabsorption, reference ranges, creatinine.*

L-Carnitine has a basal role in the facilitation and modulation of the transport of long-chain fatty acyl moieties across the inner mitochondrial membrane via a carnitine acetyltransferase system. In the last decade, clinical investigations have demonstrated that the prevalence of carnitine deficiency is relatively high, occurring as both primary[2] and secondary to other disorders[3,4] and organic acidurias[5]. Decreased carnitine concentrations have also been reported in children with Reye-like syndrome[6] and in those treated with valproic acid[7]. However, information about free, total and acylcarnitine serum and urine levels in healthy children and adults is scarce and unsatisfactory[8-12]. Some studies

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have been concerned only with free carnitine[12-14] or total carnitine[15, 16] others have not specified whether the values given refer to free or total carnitine and used less sensitive methods[12]. The relationships between esterified and free carnitine in serum and urine (AC/FC ratio) are sensitive indicators of metabolic derangement. In addition, the acylcarnitine/free carnitine clearance ratio (RAFCC) and the renal reabsorption rate for free carnitine (RFCC) are essential for the evaluation of the renal handling of carnitine. Data concerning the normal values of these parameters is lacking[9]. In this study we aimed to establish the normal ranges of free, total and acylcarnitines in both the serum and urine of healthy Turkish children and adults, and to assess carnitine metabolism and excretion during growth.

Material and Methods

The study group consisted of: a) 50 children aged six months to ten years who were referred for minor disturbances such as frequent infections, abdominal pain, minor injuries or enuresis; and b) 18 adult volunteers, 20 to 35 years of age. All subjects were metabolically healthy, with no cardiac, renal, gastrointestinal, hematologic or myopathic disease. Informed consent was obtained from the children's parents and from the adults. Children were divided into three groups according to age: 1. six months to two years (n = 15), three to five years (n = 19), and six to ten years (n = 16). All blood samples were drawn in the morning after an overnight fast. Samples of single voids of urine were obtained simultaneously. Serum was separated by centrifugation and, along with urine samples, stored at -20 °C until analysis. Serum and urine free, total and acylcarnitine determinations were made using a modified radio-enzymatic assay[8]. RRFC and RAFCC were calculated by the following equations:

RRFC = (1-UFCxSCr/SFCxUCr) x 100%; RAFCC = UACxSFC/SACxUFC (SFC is serum free carnitine, SAC is serum acylcarnitine, SCr is serum creatinine, UFC is urine free carnitine, UAC is urine acylcarnitine and UCr is urine creatinine).

Serum and urine creatinine concentrations were determined using an automated Jaffe method.

Statistical Analysis: For means, standard deviations and ranges, descriptive statistics were applied. One-way ANOVA and hypothesis tests for means were used to test the statistical significance between means. Linear regression was used to evaluate the relationship between age and carnitine concentrations. All statistical analyses were computed using the Microsta software program.

Results

Table I summarizes the serum data. Serum free and total carnitine were found to increase significantly until ten years of age. Serum AC and the AC/FC ratio reached adult values by the age of five years.

Table I: Serum Free, Total, Acylcarnitine and AC/FC ratios in Healthy Children and Adults

Age Groups	1	2	3	4
Age	6 months-2 years	3-5 years	6-10 years	20-35 years
SFC (μM)	35.02 ± 9.2[a] (21.3-46.1)	43.63 ± 11.9[b] (25.1-58.6)	52.8 ± 11.1[c] (31.8-66.2)	52.2 ± 11.9 (33.5-67.1)
STC (μM)	44.5 ± 11.3[d] (27.1-58.4)	52.4 ± 13.1[e] (31.9-68.6)	60.2 ± 11.0[f] (38.2-73.1)	59.6 ± 11.9 (40.9-74.5)
SAC (μM)	9.4 ± 2.1[g] (5.8-12.4)	8.3 ± 1.4[h] (6.6-10.6)	7.8 ± 1.0 (6.4-9.5)	7.6 ± 0.6 (7.4-9.3)
SAC/SFC	0.27 ± 0.02[i] (0.25-0.3)	0.21 ± 0.04[j] (0.17-0.27)	0.20 ± 0.02 (0.15-0.21)	0.19 ± 0.03 (0.14-0.22)
n	15	19	16	18

The values are expressed as mean ± SD. The ranges are shown in brackets.
Significantly lower ($p < 0.01$) a vs. b, b vs. c; Significantly lower ($p < 0.05$) d vs. e, e vs. f; Significantly higher ($p < 0.05$) g vs. h; Significantly higher ($p < 0.001$) i vs. j.

Urinary excretion of carnitines displayed a different pattern (Table II).

Table II: Urinary Distribution of Carnitines and AC/FC Ratios in Healthy Children and Adults

Age Groups	1	2	3	4
Age	6 months-2 years	3-5 years	6-10 years	20-35 years
UFC (μmol/g Cr)	35.8 ± 22.6[a] (2.4-60.2)	68.5 ± 42.4[b] (15.0-140.7)	70.6 ± 33.5 (12.6-156.1)	75.0 ± 38.6 (16.2-160.0)
UTC (μmol/g Cr)	146.6 ± 91.3 (11.0-284.6)	158.0 ± 68.6 (53.6-295.8)	175.5 ± 70.1 (48.2-331.2)	204.8 ± 66.0 (107.5-346.0)
UAC (μmol/g Cr)	110.8 ± 69.3[c] (5.8-12.4)	104.5 ± 39.4[d] (6.6-10.6)	126.8 ± 40.4[e] (6.4-9.5)	144.8 ± 35.1 (7.4-9.3)
UAC/UFC	3.2 ± 0.4[f] (2.7-3.8)	1.9 ± 0.77[g] (1.1-3.3)	2.6 ± 0.8[h] (1.1-3.5)	2.7 ± 0.7 (1.1-3.3)
n	15	19	16	18

The values are expressed as mean ± SD. The ranges are shown in brackets.
Significantly lower ($p < 0.01$) a vs. b; Significantly higher ($p < 0.05$) c vs. d; Significantly lower ($p < 0.05$) d vs. e; Significantly higher ($p < 0.001$) f vs. g; Significantly lower ($p < 0.01$) g vs. h.

Urinary free carnitine increased significantly up to five years, and a slight increase was noted thereafter. Urinary total carnitine increased insignificantly in all groups. A statistically significant decrease in urinary acylcarnitines and urinary AC/FC ratios was observed in the three- to five-years group, followed by a significant

increase in the six-to ten-years group. RRFC and RAFCC were both observed to increase significantly until five years of age. A slight increase was found in the older group up to adulthood (Table III).

Table III: Renal Reabsorption Rate for Free Carnitine (RRFC), AC/FC Clearance Ratio (RAFCC), Serum and Urine Creatinine Ranges in Healthy Children and Adults

Age Groups	1	2	3	4
Age	6 months-2 years	3-5 years	6-10 years	20-35 years
RRFC (%)	98.5 ± 0.94[a] (96.5-99.7)	99.1 ± 0.79[b] (96.3-99.7)	99.4 ± 0.31 (98.8-99.8)	99.5 ± 0.33 (98.6-99.9)
RAFCC (µmol/g Cr)	11.7 ± 1.4[c] (10.1-15.2)	15.6 ± 2.7[d] (10.7-20.6)	17.9 ± 6.0 (10.9-34.5)	18.1 ± 7.3 (10.5-41.9)
SCr (mg dl)	0.18 ± 0.07[e] (0.18-0.39)	0.32 ± 0.06[f] (0.2-0.39)	0.41 ± 0.11[g] (0.25-0.6)	0.5 ± 0.1[h] (0.3-0.65)
UCr (mg/dl)	18.1 ± 6.7[i] (8.0-29.1)	69.6 ± 49.2[j] (18.0-165.0)	76.2 ± 28.7[k] (28.0-120.0)	112.8 ± 41.0[l] (55.0-163.0)
n	15	19	16	18

The values are expressed as mean ± SD. The ranges are shown in brackets.
Significantly lower ($p < 0.05$) a vs. b, g vs. h; Significantly lower ($p < 0.001$) c vs. d, e vs. f, f vs. g, i vs. j, k vs. l.

Serum creatinine (SCr) showed a statistically significant increase in all age groups.Urine creatinine (UCr) displayed the same pattern, except in the three to five-years and six-to ten-years groups, where only a slight increase was present (Table III).

Discussion

The serum free, total and acylcarnitine concentrations corresponded well with the findings of other studies[10-12]. (Table I). Discrepancies with some reports in regard to ranges[8, 12, 17-19] may be due to methodological differences and variation in the selection of age groups. A positive correlation was observed between total ($r = 0.51$) and free carnitine ($r = 0.63$) serum concentrations and the age of children. We observed free and total carnitine values to reach adult values in the six to ten-years group. This finding corroborates that of Sousa et al.[8], who reported plasma free carnitine to increase significantly in the five- to 12-year age group; this increase may be explained by the activation of hormones, maturity in hepatic synthesis or reduced oral intake of carnitine in younger age groups.

The AC/FC ratio and acylcarnitines were negatively correlated with age ($r = -0.46$). Adult values were found to be reached by five years of age. The decrease in

plasma acylcarnitines and AC/FC ratios with age has also been mentioned by other authors[8, 10]. The reason may be higher fatty acid oxidation and ketogenesis at earlier ages.

The urinary total, free and acylcarnitine concentrations compared well with those of other studies[8, 9, 19] (Table II). An age-related increase in urinary total or free carnitine excretion has been reported in previous studies [8, 12, 13, 17, 20]. We found urinary free carnitine to increase significantly up to five years of age ($r = 0.59$). Battistella et al.[12] reported free carnitine excretion to be low in the first three years of life. In the literature, a sex-related difference in the urinary free carnitine excretion pattern has been reported after adolescence, and a hormonal factor suggested[21]. The increased urinary output of free carnitine in older ages may be due to endocrine factors or to a quantitatively different muscular activity[22].

Urinary total carnitine increased slightly throughout childhood up to adult ages. Acylcarnitine excretion and the urinary. AC/FC ratio followed a different pattern (Table II), decreasing significantly in the three- to five-years group and increasing to adult values in the six- to ten-years group.

In this study, the observed RRFC in children aged three to five years (99.1 ± 0.79) (Table III) was comparable to that of Matsuda et al.[9] The relatively decreased RRFC in the younger age group, with lower serum free carnitine levels, suggests a decreased renal threshold for free carnitine. The reabsorption rate for free carnitine could be influenced by the AC/FC ratio and the amount of acylcarnitines in serum[9]. No report has mentioned the variation in RFCC andd RAFCC throughout childhood. In this study, a statistically significant increase in these two parameters was observed until five years, followed by a slight increase in the older age groups.

According to the results of this report, carnitine homeostasis seems to be restored in the age range of five to ten years. More detailed studies are needed to test this finding.

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