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Free carnitine and acetyl carnitine plasma levels and their relationship with body muscular mass in athletes

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Summary. The purpose of the present study was to investigate the relationship between plasma carnitine concentration and body composition variation in relation to muscular and fat masses since there is no experimentally proved correlation between plasma carnitine and body masses. We used bioelectric impedance analysis (BIA), to determine body composition and to have a complete physical fitness evaluation. The post-absorptive plasma free carnitine and acetyl carnitine plasma levels, body composition as Fat-Free Mass (FFM) and Fat Mass (FM) in kg, as well as in percent of body mass, were analysed in 33 healthy subjects. A significant negative correlation was found between plasma acetyl carnitine and FFM in weight (kg) as well as in percent of body mass (respectively $p < 0.0001$; $p < 0.01$); a significant positive correlation was found only between FM in percent and plasma acetyl carnitine ($p < 0.01$). The observed negative correlation between plasma acetyl carnitine and muscular mass variation might reflect an oxidative metabolic muscle improvement in relation to muscular fat free mass increment and might be evidence that muscle metabolism change is in relation to plasma acetyl carnitine concentration.

Keywords: Amino acids – Carnitine – Acetyl carnitine – Body composition – Bioelectric impedance – Muscular mass

Introduction

Carnitine is an essential factor for the transport of long-chain fatty acids into the mitochondrial matrix, where they undergo β-oxidation (Bremer, 1990). Carnitine is present in tissues and fluids in free and esterified forms, the latter as short-chain and long-chain acyl carnitines; the predominant short-chain acyl carnitine is acetyl carnitine. In humans at rest, acetyl carnitine accounts for 10 to 30% of the total carnitine in plasma and 5 to 40% of the total carnitine in the muscle and liver tissues. 70–80% of total carnitine in urine is acetyl carnitine. These proportions vary with nutritional and exercise states. Free carnitine

appears to act like a buffer in individuals with high metabolic rate and it functions as an acceptor of accumulating acetyl groups. Acetyl carnitine moves freely across subcellular membranes and acts as a pool from which acetyl-CoA is regenerated. This makes it possible for metabolic energy to be transported between different organs. Changes in plasma carnitine (free and acetylated), observed during exercise, as in other conditions (e.g. fasting, diabetes), could result from an interorgan exchange and a turnover may occur between plasma carnitine and liver carnitine pools such as between muscle and plasma. Diet, endogenous synthesis and efficient re-absorption by the kidney influence plasma carnitine levels; furthermore carnitine baseline levels change with sex, age and nutritional status (Stadleer et al., 1993; Guder and Wagner 1990). The total carnitine pool in a normal individual is about 100 mmol, the major pool is in the skeletal muscle (98%: of which approximately 77% is in the free form and 19% is acetyl carnitine), the remaining (1.5%) is in the liver and in the kidney (Engel and Rebouche 1984). Carnitine turnover is faster in the liver and in the kidney than in skeletal muscle in rats (Brooks and McIntosh 1975) Rebouche et al. (1993) observed in vegetarians that the kidney adapts to carnitine intake by reducing the efficiency of carnitine re-absorption. Carnitine dietary supplementation was used to enhance human exercise performance, supporting the hypothesis that carnitine plays an essential role in the regulation of long-chain fatty acid oxidation in skeletal muscle (Cerretelli and Marconi, 1990; Arenas et al., 1991; Wyss et al., 1990; Siliprandi et al., 1992). Many experimental researches in this matter have demonstrated that carnitine supplementation does not influence lipid and carbohydrate oxidation during exercise and conclude that in both healthy human athletes and sedentary subjects, carnitine supplementation does not influence muscle substrate utilisation either at rest or during prolonged exercise (Vukovich et al., 1994; Brass et al., 1994; Trappe et al., 1994). Spagnoli et al. (1990) observed a trophic effect of L-carnitine supplementation on human skeletal muscle. In regard to the carnitine and acetyl carnitine role in oxidative and energetic metabolism, very few studies have explored the relationship between body composition and plasma carnitine, Amodio et al. (1990) found no relationship between triceps skinfold and carnitine plasma levels or carnitine esterification ratios. The parameters related to body composition and the techniques used to study it, are various; bioelectric impedance analysis (BIA) and the anthropoplicometric method are the major non-invasive methods used (Kabir et al., 1994; Rabeneck et al., 1993; Royal et al., 1994).

The aim of the present study was to investigate the relationship between plasma carnitine and acetyl carnitine levels and body composition, measured in post-absorptive state by BIA.

Materials and methods

Subjects

The study comprised a group of 33 well trained athletes (26 males and 7 females). Their age range was 14–49 years, height 164–185.5 cm, body weight 48–81 kg, and body mass index 17.8–25.5 $kg \cdot m^{-2}$. Table 1 summarises the physical characteristics of the study

Table 1. Physical characteristics

Characteristics	Males (n = 26)	Females (n = 7)	Total (n = 33)
Age (years)	30.2 (8.8)	14.6 (0.5)	26.9 (10.2)
Height (cm)	174.9 (5.0)	169.7 (6.1)	173.8 (5.6)
Body weight (kg)	70.4 (6.3)	59.4 (7.0)*	68.1 (7.8)
BMI ($kg \cdot m^{-2}$)	23.0 (1.4)	20.6 (1.4)*	22.5 (1.73)
FFM (kg)	60.2 (5.8)	45.2 (4.8)*	57.0 (8.3)
FFM (%)	85.6 (2.3)	76.3 (3.1)*	83.6 (4.7)
FM (kg)	10.2 (2.3)	14.2 (2.8)*	11.0 (2.8)
FM (%)	14.4 (2.8)	23.2 (2.7)*	16.3 (4.6)

Values are means (SD). * $p < 0.0001$ males vs females. All female subjects were in fertile age.

subjects. All subjects were on a weight – maintaining diet of which the caloric content was distributed as 60% carbohydrates, 25% fats and 15% proteins, without alcohol intake, and 22 (males) followed a triathlon (cycling, swimming and running) and the remaining 11 (males n = 4, females n = 7), swimming sports. The athletes were informed of the nature, purpose and protocol details of the experiment before their consent was obtained.

Methods

Blood samples were drawn from ante cubital vein into heparinate tubes, after a four hour fast, in order to assay post-absorptive plasma free carnitine and acetyl carnitine levels.

Blood was centrifuged, and plasma was stored at −20°C until analysis.

Carnitine and acetyl carnitine concentrations were measured, after filter separation (Amicon – Centricon™ 30), by enzymatic spectrophotometric and fluorimetric assay, respectively, as previously described (De Palo et al., 1993).

Briefly 2 mL of plasma was filtrated with 30 kDa cut-off filter to obtain 800 μL of filtrate: 150 μL was used for carnitine assay, by enzymatic-colorimetric method with 5,5′-dithiobis (2-nitro-benzoic) acid (DTNB), and 500 μL was used for acetyl carnitine assay by enzymatic fluorimetric method.

With regard to the plasma free carnitine assay, the intraserial imprecision calculated (using replicated analysis) from duplicate estimation of a patient sample was 2%; the coefficient of the day to day variation, similarly calculated, was 6%. The mean concentration of healthy subjects was 34 ± 1 nmol/mL (mean ± sem, n = 24). The assay has a minimum detection limit of 2 nmol/mL estimated from 3 standard deviation of a standard sample without carnitine.

With regard to the acetyl carnitine assay, the intraserial imprecision calculated from duplicate estimation of a patient sample, was 10%; the coefficient of the day to day variation, similarly calculated, was 8%. The mean concentration of healthy subjects was 4.3 ± 0.3 nmol/mL (mean ± sem, n = 24). The assay has a minimum detection limit of 0.2 nmol/L estimated from 3 standard deviation of a standard sample without acetyl carnitine.

Body composition, such as Fat-Free Mass (FFM) and Fat Mass (FM) in kg and in percent of body mass, was measured in post-absorptive state by Bioelectric Impedance Assay (BIA).

Bioelectric Impedance (Z) measurement was carried out with the subjects supine using a four terminal impedance plethysmograph (Model BIA – 109, RJL Systems, Detroit, MI, USA), according to the manufacturer's instructions. Briefly, resistance (R) and reactance (Xc) (in ohms) were measured as the device passed a 800 μA current between surface electrodes applied to the skin of the dorsum of the right hand and foot. The measurement was painless and completed in one minute. A computer calculated body composition parameters [FFM, FM, TBW (Total Body Water)] by derived equa-

tions using the resistance and reactance measurements ($Z = \sqrt{R^2 + Xc^2}$ and $V = \varrho L^2/Z$ where V can be assumed to be the volume of TBW, ϱ is the specific resistivity and L length equal to body height) together with subject's age, weight and height. FFM was obtained from TBW, assuming that the hydratation of FFM is 0.73. FM was obtained from the difference between body mass and FFM. Mean reference values of FFM and FM in percent in our laboratory were respectively 74.9 ± 3.95% and 25.04 ± 3.95% for healthy female subjects (n = 53) and 83.60 ± 4.11% and 16.39 ± 4.11% for healthy male (n = 63) subjects.

Body Mass Index (BMI) was calculated as the ratio between body weight (kg) and the square of the height (m).

Statistical analyses

The results are given as mean ± Standard Deviation (SD) where values are not expressed differently. Descriptive statistics and Student's test were used where appropriate. Linear regression and correlation coefficients were calculated by the conventional least square method and expressed with 95% confidence limits. Statistical significance was $p < 0.05$.

Results

Plasma free carnitine and acetyl carnitine average concentrations were 40 ± 1 nmol/mL and 3.4 ± 0.5 nmol/mL (means ± sem) respectively in all subjects. There was a significant difference between males (M) and females (F) in plasma carnitine (M = 41 ± 1 nmol/mL and F = 35 ± 2 nmol/mL, means ± sem) and acetyl carnitine (M = 2.8 ± 0.4 nmol/mL and F = 7.2 ± 0.8 nmol/mL, means ± sem) $p < 0.02$ and $p < 0.0001$ respectively. Females had a significantly ($p < 0.0001$) higher acetyl/free ratio than males (M = 0.71 ± 0.01 and F = 0.20 ± 0.02, means ± sem). There was, as expected, a significant difference (see Tab. 1) between males and females in relation to FFM (% and kg), Fat (% and kg) and BMI. The relationship between FFM in kg and in percent of body mass respectively and plasma acetyl carnitine is reported in Fig. 1 and Fig. 2, the regression line for all the subjects is shown.

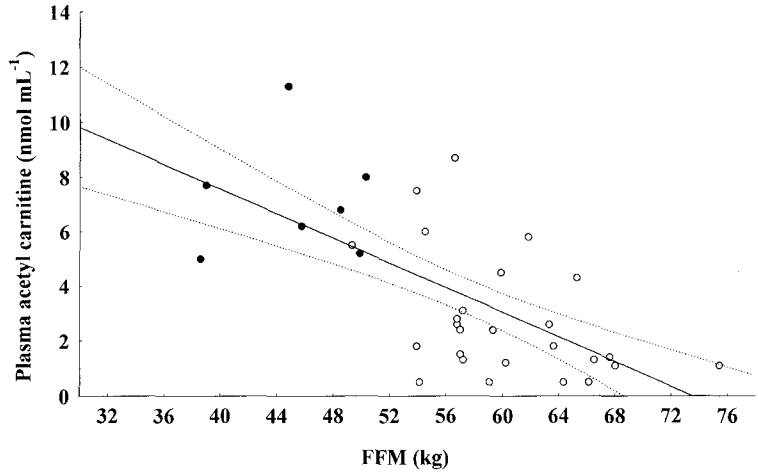

Fig. 1. Relationship between plasma acetyl carnitine and FFM in kg of body weight. Dotted line 95% confidence limits. Dark points indicate female subjects

The parameters of the regression analysis were as follows: Fig. 1: $y = -0.23x + 16.59$, $r = 0.65$, $p < 0.0001$; Fig. 2: $y = -0.26x + 25.57$, $r = 0.43$, $p < 0.01$. Fig. 3 shows the relationship between FM in percent and plasma acetyl carnitine, and the parameters of the regression analysis demonstrated the following equation: all subjects: $y = 0.28x - 0.79$, $r = 0.44$, $p < 0.01$. The correlation between FM in kg and plasma acetyl carnitine was as follows: $y = 0.19x + 1.58$, $r = 0.19$, $p =$ n.s. (Fig. 4).

The plasma free carnitine and FM and FFM in kg and in percent demonstrated no statistical correlation. The correlation between BMI and acetyl carnitine was $y = -1.18x + 30.2$, $r = 0.71$, $p < 0.0001$ (Fig. 5), with no statistical correlation between free carnitine. In regard to the correlation between different sexes and FFM, FM and BMI there was significant correla-

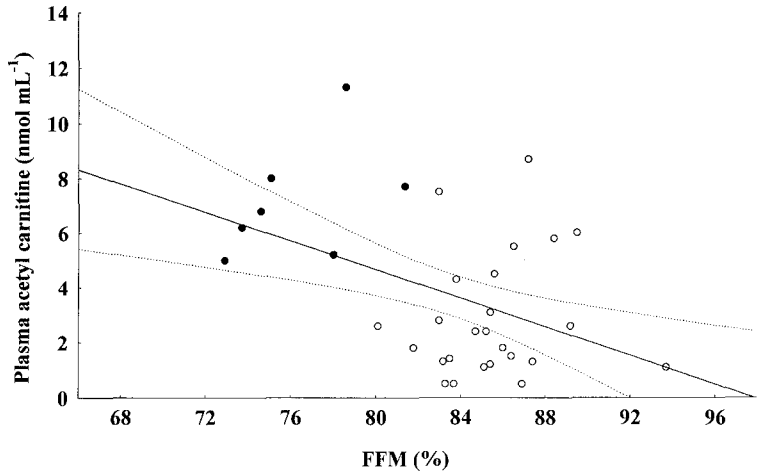

Fig. 2. Relationship between plasma acetyl carnitine and FFM in percent of body weight. Dotted line 95% confidence limits. Dark points indicate female subjects

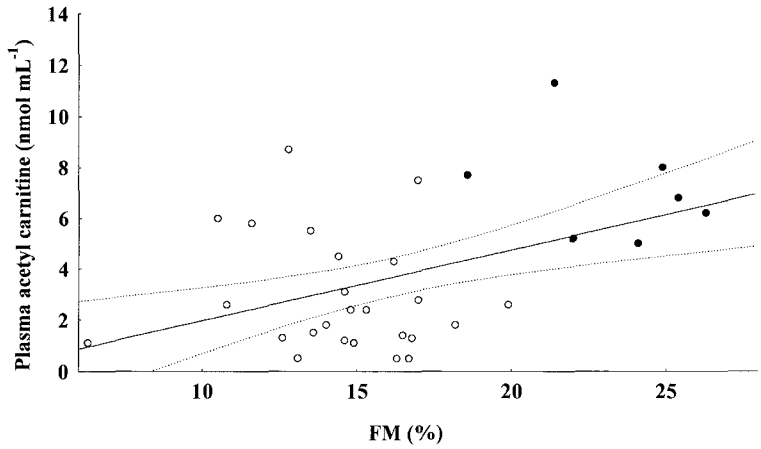

Fig. 3. Relationship between plasma acetyl carnitine and FM in percent of body weight. Dotted line 95% confidence limits. Dark points indicate female subjects

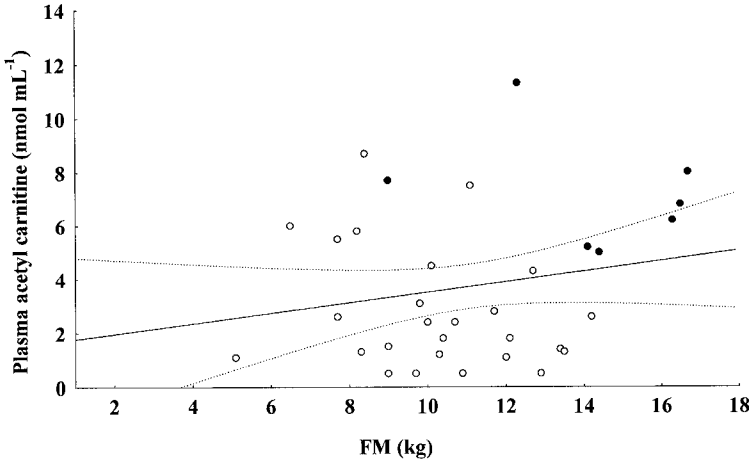

Fig. 4. Relationship between plasma acetyl carnitine and FM in kg of body weight. Dotted line 95% confidence limits. Dark points indicate female subjects

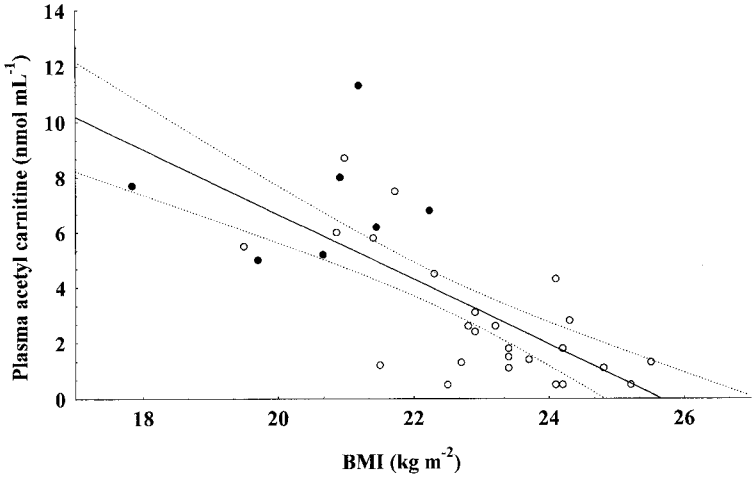

Fig. 5. Relationship between plasma acetyl carnitine and BMI. Dotted line 95% confidence limits. Dark points indicate female subjects

tion only in males between acetyl carnitine and FFM in kg and BMI ($p < 0.0001$ and $p < 0.00001$ respectively). No correlation was found in the females only.

Discussion

Carnitine and acetyl carnitine plasma concentrations obtained in present and other work in our laboratory, confirmed that in humans free plasma carnitine levels are higher in males than in females, in agreement also with other authors. Plasma acetyl carnitine and acetyl carnitine/free carnitine ratio are higher in females than in males, this observation was also confirmed by

Lambert et al. (1988). An explanation of this might be in relation to their different muscular masses as demonstrated also in our laboratory (74.9 ± 3.95% of body mass for healthy female subjects (n = 53) and 83.60 ± 4.11% of body mass for healthy male subjects (n = 63), as confirmed by other authors (Leiter et al., 1994; Heywar D, 1996) Furthermore we found, in all subjects, a negative correlation between plasma acetyl carnitine and FFM in percent as well as in kg of body mass, and a positive correlation between FM in percent of body mass. No correlation in the female subjects was found, but in the male subjects a significant negative correlation between acetyl carnitine plasma levels and FFM in kg of body mass and BMI was observed. As expected a significant difference between males and females in relation to FFM (% and kg), Fat (% and kg) and BMI , was present. Harper et al. (1995) found that muscle free carnitine concentration was significantly higher and muscle acyl carnitine was lower in obese women characterised by an enlarged fat mass than in control subjects. Boulat et al. (1993) showed that females with BMI >28 had a lower plasma acyl/free carnitine ratio than in the female group with BMI < 28. Carnitine muscle concentration variations are related to the muscle metabolic state as demonstrated by Cederblad et al. (1974), but a correlation between the levels of plasma carnitine, such as its esters, and their concentrations in the muscular tissue, was not found. However not all literature observations in this matter are in agreement. An unclear relationship between the muscle levels of free and total carnitine and the levels of plasma carnitine was indicated by Starling et al. (1995) and for this author the measurements of plasma carnitine seem unable to represent the carnitine status of healthy subjects. Brass and Hiatt (1994) and De Palo et al. (1993) suggested that plasma acetyl carnitine levels reflect carnitine metabolism variations better than free and total carnitine in human plasma. Many researchers have demonstrated that exercise increases acetyl carnitine concentration in the muscle furthermore its plasma variation seems in relation to the work load, in addition plasma free carnitine levels do not vary so clearly (Brass and Hiatt, 1994). Hiatt et al. (1990) suggested that exercising muscle metabolism improvement was in relation to chronic changes in muscular acetyl carnitine concentration; these variations were also measured in the plasma levels. Spagnoli and co-workers (1990) observed that long-term administration of L-carnitine affects human muscle fibers with a specific trophic effect on type 1 fibers, which are characterised by an oxidative metabolism, and a muscle carnitine concentration relation with the oxidative potential of the muscle was suggested (Cederblad et al., 1976).

In regard to body composition it is known that body mass variation is in relation to different physiopathological conditions. A body composition analysis was used in the present study and the amount of fat mass and fat free mass as well as body mass were measured. The correlation between BIA, anthropometry and hydro-densitometry in relation to fat mass has also been studied by many other authors and BIA gives a good estimation of body mass variations (Lukaski et al., 1985; Schols et al., 1987).

The relation between plasma acetyl carnitine and body composition expressed as FFM and FM, measured by BIA in all the subjects in present study,

might be explained partially by the dependence of plasma acetyl carnitine levels on fat oxidation (in tissues), particularly if we consider that plasma acetyl carnitine concentration depends in part on muscular uptake or better on turnover linked to muscular metabolic state and in part on the exchange processes between blood and other tissues like the liver. Therefore the observed correlation could reflect a metabolic muscle improvement in agreement with Hiatt et al. (1990, 1996) in relation to muscular fat free mass increment.

Further studies are needed in order to define this parameter as an indicator of muscle mass size and perhaps as a potential evaluation of the ability in sustaining exercise.

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