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Serum total L-carnitine levels in non-obese women with polycystic ovary syndrome

Semin Melahat Fenkci[1,6], Veysel Fenkci[2], Ozer Oztekin[3], Simin Rota[4] and Nedim Karagenc[5]

[1]Department of Internal Medicine, Division of Endocrinology and Metabolism, Pamukkale University, School of Medicine, 20100 Denizli, Turkey; [2]Clinics of Obstetrics and Gynaecology, Special Aegean Hospital, 20100 Denizli, Turkey; [3]Departments of Obstetrics and Gynaecology, Pamukkale University, School of Medicine, 20100 Denizli, Turkey; [4]Department of Biochemistry, Pamukkale University, School of Medicine, 20100 Denizli, Turkey; [5]Department of Medical Biology, Pamukkale University, School of Medicine, 20100 Denizli, Turkey

[6]Correspondence address: Zeytinkoy Mah. Dogalevler Sitesi 5097 Sokak, M Blok kat: 2 Daire: 4 Bagbasi, 20100 Denizli, Turkey. Tel: +90-505-400-40-27; Fax: +90-258-264-01-52; E-mail: sfenkci@yahoo.com

BACKGROUND: Carnitine plays essential roles in energy production, oxidative stress and glucose metabolism. This study was planned to determine serum total L-carnitine levels in non-obese women with polycystic ovary syndrome (PCOS). METHODS: There were 27 non-obese women with PCOS and 30 healthy, age- and body mass index (BMI) matched controls were evaluated in this controlled clinical study. Serum lipid sub-fractions, fasting glucose, insulin and other hormones (gonadotrophins, androgens) and total L-carnitine levels were measured. Homeostasis model assessment (HOMA-IR) was used to estimate insulin resistance. RESULTS: The women with PCOS had significantly higher serum dehydroepiandrosterone sulfate, total testosterone, free androgen index (FAI), luteinizing hormone (LH), low-density lipoprotein (LDL) cholesterol, non-high density lipoprotein (HDL) cholesterol, fasting insulin levels and HOMA-IR measurement and LH/FSH ratios than healthy women. However, total L-carnitine and sex hormone-binding globulin (SHBG) levels were significantly lower in women with PCOS. L-Carnitine level was negatively correlated with FAI, but positively correlated with SHBG. Multiple regression analysis revealed that SHBG was a strong predictor of serum total L-carnitine level. CONCLUSIONS: Decreased total L-carnitine levels may be associated with hyperandrogenism and/or insulin resistance in non-obese women with PCOS. Long-term studies are needed to evaluate carnitine metabolism in PCOS, especially with regard to the molecular basis.

Keywords: carnitine; PCOS; insulin resistance; glucose metabolism; hyperandrogenism

Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous endocrine disorder, affecting ~7% of reproductive age women (Asuncion *et al*., 2000), and is characterized by chronic anovulation, hyperandrogenism and disordered gonadotropin secretion (Dunaif, 1997). Its clinical and biochemical manifestations may include oligo-amenorrhea, obesity, enlarged cystic ovaries, elevated luteinizing hormone (LH), signs of androgen overproduction and reduced fertility. Insulin resistance plays pivotal roles in the pathogenesis of PCOS (Arslanian *et al*., 2001), even though the mechanisms underlying PCOS are not completely understood. The presence of hyperinsulinemia in patients with PCOS, independent of obesity, was previously confirmed (Dunaif *et al*., 1989). Hyperinsulinemia occurs as a result of insulin resistance and accelerates ovarian androgen overproduction (Dunaif *et al*., 1992). Hyperinsulinemia may also contribute to the development of diabetes and dyslipidemia in PCOS patients (Dunaif, 1997). We have previously shown that there is increased oxidative stress and decreased antioxidant capacity in patients with PCOS (Fenkci *et al*., 2003).

Carnitine plays an essential role in fatty acid metabolism by facilitating the transport of long-chain free fatty acids into the mitochondrial matrix, making them available for β-oxidation, which is the most efficient metabolic pathway for energy production (Borum, 1980). Carnitine also plays a key role in glucose metabolism and in fuel-sensing, because it behaves as a shuttle for acetyl groups from inside to outside the mitochondrial membrane. Accumulation of acyl-CoA and acetyl-CoA compounds inside the mitochondria is implicated in the pathogenesis of lipid-induced insulin resistance (Mingrone, 2004; Power *et al*., 2007). Carnitine has also an antioxidant capacity and improves oxidative stress (Mister *et al*., 2002; Gómez-Amores *et al*., 2006).

This study was planned to determine serum total L-carnitine levels in non-obese women with PCOS. To the best of our

knowledge, we present the first study concerning serum total carnitine levels in PCOS.

Materials and Methods

Subjects

There were 27 non-obese patients with PCOS (study group) and 30 healthy women (control group) included in the study. The patients' ages ranged from 16 to 37 years. The diagnosis of PCOS was based on the recommendations of Rotterdam consensus conference on PCOS by at least two of the following three features: (i) oligo- or anovulation, (ii) clinical and/or biochemical signs of hyperandrogenism and (iii) polycystic ovaries (The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004). All subjects in study group had clinical hyperandrogenism [presence of acne, Ferriman–Gallwey score of $\geq$8] (Ferriman and Gallwey, 1961), oligomenorrhea (fewer than six menstrual periods in the preceding year) and the presence of 12 or more subcapsular follicles in each ovary measuring 2–9 mm in diameter by transvaginal ultrasound examination. Exclusion criteria comprised infectious diseases, hypertension, family history of cardiovascular disease, chronic liver diseases, endocrinopathies including diabetes, Cushing's syndrome, androgen secreting tumors, non-classical 21-hydroxylase deficiency, thyroid dysfunction, hyperprolactinemia, smoking, alcohol consumption and use of all medications known to alter sex hormones, lipoprotein, carnitine metabolism, or insulin secretion or action.

The women in the control group had regular menstrual cycles that were defined as cyclic uterine bleedings with duration of 4–5 days and a frequency of 25–34 days. The healthy state of control subjects was determined by medical history, physical and pelvic examination, and whole blood chemistry. None of the 30 healthy women in the control group met any exclusion criteria mentioned above. This work was approved by the local medical ethics committee and all participants gave informed consent before the onset of study.

Biochemical analysis

Venous blood samples were drawn from all participants after fasting for 12 h on the study day (on cycle days 3–5 after spontaneous or progesterone-induced menses in the PCOS and control groups). Samples were collected in serum separator tubes, allowed to clot for 30 min, centrifuged for 15 min at 2000 $\times$ *g* at room temperature and aliquoted into polypropylene tubes. All biochemical measurements were performed on same day except L-carnitine. For total L-carnitine measurements, the aliquoted samples were stored in -20°C until analysed. Biochemical measurements were done by using commercial kits. The sera were assayed for fasting glucose (F.Glc), triglyceride (TG), total cholesterol (TC), HDL cholesterol, insulin, dehydroepiandrosterone sulfate (DHEA-S), sex hormone-binding globulin (SHBG), follicle-stimulating hormone (FSH), LH, total testosterone and total L-carnitine. Serum glucose (hexokinase), TC (CHOD-PAP) and TG (GPO-PAP) concentrations were measured using standard enzymatic methods (Roche Diagnostics, IN, USA) with a fully automated analyser (Roche Modular PE, Roche Diagnostics). HDL-cholesterol concentrations were measured without precipitation by using liquid selective detergent homogeneous technique (Roche HDL-C plus 2nd generation, Roche Diagnostics). LDL levels were calculated by using Friedewald's formula. Non-HDL cholesterol levels were computed by the formula: non-HDL = TC − HDL. Fasting insulin, FSH, LH and total testosterone concentrations were measured using electrochemiluminescence's immunoassay (Roche Diagnostics) with a fully automated analyser (Roche Modular PE, Roche Diagnostics). SHBG, DHEA-S immunometric assays were performed using a solid phase competitive chemiluminescence's immunoassay (IMMULITE 2000, DPC Biosystems, CA, USA).

FAI was defined here as 100 times the molar ratio of total testosterone to SHBG [FAI = 100 $\times$ total testosterone (in nmol/l)/SHBG (in nmol/l)].

Insulin resistance was calculated by using homeostasis model assessment (HOMA-IR) score that employs the formula: fasting insulin concentration (mIU/l) $\times$ glucose (mmol/l)/22.5 (Matthews *et al*., 1985).

For total L-carnitine measurements, all samples of the subjects were assessed during a single assay run. The measurements were performed using a commercial kit (Roche Diagnostics GmbH, Penzberg, Germany) according to the instructions of the manufacturer. The test was based on the measurement of the amount of NADH consumed during the reaction. The test was linear in the range of 5.6–112 μmol/l of L-carnitine. Samples with L-carnitine levels above the linearity range were diluted according to the instructions of the manufacturer. For biochemical measurements, the within-run CV values were ranging between 0.7 and 2.1%, and the between-run CV values were ranging between 1.2 and 2.6%.

Anthropometric measurements

All anthropometric measurements were done by the same physician on the day that the blood specimen was taken. Waist circumference (c.m.) was taken with a tape measure as the point midway between the costal margin and iliac crest in the mid-axillary line with the subject standing and breathing normally and hip c.m. was measured at the widest point around greater trochanter. Body mass index (BMI) (Body weight (kg)/height m^2) and waist-to-hip ratio (WHR) were computed.

Statistical analysis

At the beginning of the study, all study participants were matched for age and BMI. The healthy controls were defined as age- and BMI-matched with subjects when the differences in age and BMI were less than 2 years and less than 1 kg/m^2, respectively. Since many variables had a Gaussian distribution with no significant skewness, statistical analysis was performed with a parametric test: Student's *t*-test. Correlations between variables were calculated with Pearson's correlation coefficient. Stepwise multiple regression analysis introducing the serum total L-carnitine level as a dependent variable and androgens, SHBG and HOMA-IR as independent variables ($r = 0.610$, $r^2 = 0.373$, Adjusted $r^2 = 0.361$, Durbin–Watson = 2.266, $F = 32.663$, $P = 0.0001$) was also conducted. After each addition of a new independent variable to the equation, all previously entered independent variables were checked to see whether they maintained their level of significance. Previously entered independent variables were retained in the regression equation only if their removal would have caused a significant reduction in r^2. The data are expressed as means $\pm$ SE. Statistical significance was set at $P < 0.05$. Data were analysed with the SPSS (Statistical Package for the Social Science, version 11.0).

Results

There were no statistically significant differences in the waist measurements, WHR and serum FSH, F.Glc, TC and TG and HDL levels between the groups. Ferriman–Gallwey score, serum LH, total testosterone, DHEA-S, fasting insulin, LDL levels and LH/FSH ratio, FAI and HOMA-IR were significantly higher in patients with PCOS compared with controls (for each parameter $P < 0.05$). However, the women with PCOS had significantly lower serum SHBG and total

L-carnitine levels than did healthy subjects (for each parameter; $P < 0.05$) (Tables I and II).

BMI was positively associated with waist measurement ($r = 0.40$, $P = 0.002$). Serum total L-carnitine level was negatively correlated with FAI ($r = -0.32$, $P = 0.016$), but positively with SHBG ($r = 0.61$, $P = 0.0001$). FAI was positively associated with LH ($r = 0.62$, $P = 0.0001$), LH/FSH ratio ($r = 0.50$, $P = 0.0001$), total testosterone ($r = 0.70$, $P = 0.0001$) DHEA-S ($r = 0.31$, $P = 0.017$), LDL ($r = 0.28$, $P = 0.033$), non-HDL ($r = 0.34$, $P = 0.011$), fasting insulin ($r = 0.53$, $P = 0.0001$), HOMA-IR ($r = 0.51$, $P = 0.0001$), and negatively associated with SHBG ($r = -0.61$, $P = 0.0001$). SHBG was negatively correlated with LH/FSH ratio ($r = 0.50$, $P = 0.0001$), HOMA-IR ($r = -0.36$, $P = 0.005$) and fasting insulin ($r = -0.40$, $P = 0.003$). On the other hand, there was a positive relationship between HOMA-IR and TG ($r = 0.42$, $P = 0.001$). As seen above, the degrees of relationships between many variables were statistically significant yet small, except the association between FAI and LH which was strong. Moreover, the correlation between serum total L-carnitine level and SHBG was strong. Stepwise multiple regression analysis introducing the serum total L-carnitine level as a dependent variable and androgens, SHBG and HOMA-IR as independent variables revealed that SHBG was a strong predictor of serum total L-carnitine level ($\beta = 0.610$, $P = 0.0001$) (Table III).

Discussion

Carnitine is a trimethylamine molecule, which plays an important role in cell energy metabolism. Carnitine is synthesized primarily in the liver and kidney from lysine and methionine (Bremer, 1983), but additional intestinal resorption is necessary (Sealey and Laragh, 1975). Carnitine facilitates energy availability, and is particularly vital for those tissues with high energy requirements such as cardiac and skeletal muscles. These tissues are the major storage sites of carnitine. Carnitine mediates the transport of activated acyl residues via the carnitine palmitoyl transferase system into mitochondria for β-oxidation (Bremer, 1983; Sealey, 1991). Carnitine also acts as a carrier of acetyl groups from mitochondria to the cytosol (Lysiak *et al*., 1988; Broderick *et al*., 1992), so that it maintains sufficient amounts of free CoA for mitochondrial function and protects mitochondria by reducing accumulation of long-chain acylcarnitine in the mitochondrial space. Long-chain acylcarnitine has a detergent effect on the mitochondrial membrane. The myocardium utilizes both glucose and fatty acids for energy metabolism. In the current study, we found decreased levels of total L-carnitine in non-obese women with PCOS. It may therefore be assumed that the energy needed for cardiac contraction is not sufficiently supplied in subjects with PCOS, since decreased levels of carnitine may limit the availability of fatty acids in the mitochondria to generate ATP.

The carnitine system is important in determining body composition, because it plays a critical role in insulin regulation of fat and glucose metabolic rate in skeletal muscle (Reda *et al*., 2003). The mechanism of carnitine action includes an increase in glucose metabolism via stimulating glucose disposal and oxidation (De Gaetano *et al*., 1999; Sethi *et al*., 1999). Also carnitine may improve insulin sensitivity in insulin resistance (Mingrone *et al*., 1999). On the other hand, above a threshold level, hyperinsulinemia causes carnitine accumulation in muscle cells via insulin-mediated stimulation of muscle carnitine transport leading to a decrease in total serum carnitine

Table I. Clinical features and steroid levels for the healthy controls and the women with PCOS.

Variable	Women with PCOS ($n = 27$)	Healthy controls ($n = 30$)	P
Age (years)	25.0 ± 1.42	26.1 ± 0.6	0.46
BMI (kg/m^2)	24.7 ± 0.6	23.5 ± 0.6	0.15
Waist (c.m.)	80.4 ± 0.9	79.0 ± 1.3	0.40
Waist/hip ratio	0.78 ± 0.01	0.79 ± 0.01	0.35
FSH (mIU/ml)	6.70 ± 0.40	5.9 ± 0.4	0.22
LH (mIU/ml)	14.7 ± 1.0	6.2 ± 0.7	0.0001[a]
LH/FSH ratio	2.3 ± 0.2	1.2 ± 0.1	0.0001[a]
Total testosterone (nmol/l)	2.33 ± 0.2	1.34 ± 0.1	0.0001[a]
SHBG (nmol/l)	21.8 ± 1.6	75.1 ± 7.9	0.0001[a]
FAI	11.3 ± 0.9	2.2 ± 0.2	0.0001[a]
DHEA-S (μmol/l)	5.1 ± 0.4	3.8 ± 0.4	0.02 [a]

[a]$P < 0.05$ statistically significant. BMI, body Mass Index; FSH, follicle-stimulating hormone; LH, luteinizing hormone; FAI, free androgen index; SHBG, sex hormone-binding globulin; DHEA-S, dehydroepiandrosterone sulfate.

Table II. Metabolic characteristics and total L-carnitine levels for the healthy controls and the women with PCOS.

Variable	Women with PCOS ($n = 27$)	Healthy controls ($n = 30$)	P
Fasting glucose (mmol/l)	5.2 ± 0.1	4.9 ± 0.1	0.11
Fasting insulin (μIU/ml)	18.0 ± 2.6	6.6 ± 0.5	0.0001[a]
HOMA-IR	4.2 ± 0.7	1.5 ± 0.1	0.0001[a]
Total cholesterol (mmol/l)	5.1 ± 0.2	4.6 ± 0.2	0.09
Triglyceride (mmol/l)	1.2 ± 0.1	1.1 ± 0.1	0.86
HDL (mmol/l)	1.3 ± 0.1	1.5 ± 0.1	0.19
LDL (mmol/l)	3.2 ± 0.2	2.5 ± 0.1	0.009[a]
Non-HDL (mmol/l)	3.8 ± 0.2	3.1 ± 0.2	0.02[a]
Carnitine (μmol/l)	40.5 ± 5.7	91.1 ± 15.2	0.004[a]

[a]$P < 0.05$ statistically significant. HOMA-IR, homeostasis model assessment; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table III. Stepwise multiple regression analysis with serum L-carnitine level as the dependent variable.

	β	P
Total testosterone (nmol/l)	0.033	0.766
SHBG (nmol/l)	0.610	0.0001[a]
FAI	0.087	0.522
DHEA-S (μmol/l)	0.101	0.361
HOMA-IR	0.056	0.630

[a]$P < 0.05$ statistically significant. SHBG, sex hormone-binding globulin; FAI, free androgen index; DHEA-S, dehydroepiandrosterone sulfate; HOMA-IR, homeostasis model assessment.

levels (Stephens *et al*., 2007). Independent of obesity, the presence of hyperinsulinemia in PCOS was confirmed by the outcomes of this study. We found an obviously higher level of fasting insulin and HOMA-IR in subjects with PCOS than in healthy controls, although BMI was in normal range in each group. Despite low levels of serum total L-carnitine in patients with PCOS, there were not any significant correlation between serum total L-carnitine levels and insulin resistance in this study. The requirement of a threshold level for an insulin effect on the carnitine level may have obscured a direct correlation.

Dyslipidemia is frequently accompanied by decreased HDL, and increased TC, LDL, TG levels and can be observed in insulin resistance conditions (DeFronzo and Ferrannini, 1991). It has been shown that carnitine has a hypotriglyceridemic effect (Gómez-Amores *et al*., 2006). Moreover, carnitine supplementation may relieve lipid overload (Power *et al*., 2007). We did not observe any relationship between serum total carnitine level and lipid fractions. But significantly elevated LDL and non-HDL levels in subjects with PCOS were found in this study. TC and TG levels were slightly higher, whereas the HDL level was lower in patients with PCOS; however, there were no significant differences in these lipid fractions between both groups possibly due to the selection of subjects who had normal BMI.

Oxidative stress is one of the major reasons of molecular damage to cellular structures and is implicated in the pathogenesis of the atherosclerosis, diabetes mellitus or ischemia-reperfusion injury (Betteridge, 2000). Carnitine improves repair mechanisms for oxidative stress-induced damage to membrane phospholipids (Sethi *et al*., 1999), and also maintains general antioxidant status (Savitha and Panneerselvam, 2006). It protects cells from reactive oxygen species (Mister *et al*., 2002; Gómez-Amores *et al*., 2006) by acting as a free radical scavenger (Vanella *et al*., 2000; Arockia Rani and Panneerselvam, 2001). Also carnitine reduces *trans*-cardiac endothelin concentrations (Lango *et al*., 2005), and enhances endothelium-dependent relaxations (Mauriello *et al*., 1996; Cipolla *et al*., 1999), therefore it partially improves vascular reactivity (Irat *et al*., 2003). Carnitine diminishes the ischemia-induced apoptosis as well (Vescovo *et al*., 2002; Ferrari *et al*., 2004). Previously, we reported increased oxidative stress and decreased antioxidant capacity in patients with PCOS (Fenkci *et al*., 2003). These observations suggest that low levels of antioxidant carnitine may further contribute to the harmful effects of increased oxidative stress in non-obese women with PCOS.

Serum carnitine levels may be influenced by androgens and estrogens (Borum, 1980). Total and free carnitine levels are lower in women than men (Chiu *et al*., 1999; Opalka *et al*., 2001), because estrogen reduces serum free carnitine levels (Takiyama and Matsumoto, 1998). In addition, androgens may induce an increase in serum carnitine concentrations (Borum, 1980). It was observed that carnitine levels were correlated with DHEA-S levels and DHEA-S could modulate carnitine levels (Chiu *et al*., 1999). In our investigation, we found increased clinical and biochemical signs of hyperandrogenism, but there was decreased serum total L-carnitine level in women with PCOS. Also the serum total L-carnitine level was negatively correlated with FAI, but positively correlated with SHBG. On the other hand, HOMA-IR was inversely correlated with SHBG, but positively correlated with FAI. Multiple regression analysis revealed that only SHBG was a strong predictor of serum the total L-carnitine level. These outcomes were in conflict with the observations mentioned above. We assume that both androgen excess and insulin resistance, being inversely related to SHBG, may have an effect in the same direction, that is, they may both influence carnitine levels negatively.

In conclusion, the outcomes of this study suggest that decreased serum total L-carnitine levels may be related with hyperandrogenism and/or insulin resistance in non-obese women with PCOS. In terms of carnitine activity, insulin resistance, oxidative stress and dyslipidemia, long-term studies are needed in PCOS, especially with regard to the molecular basis of the interactions.

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