Plasma L-carnitine levels of obese and non-obese polycystic ovary syndrome patients

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ORIGINAL ARTICLE

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ABSTRACT

It is well-known that plasma L-carnitine concentrations are significantly decreased in obese individuals. A study showed that L-carnitine concentrations are significantly lower in lean PCOS patients than in lean healthy women. Thus, it has been suggested that lowered L-carnitine is associated with PCOS. This study also showed that the women with PCOS had significantly lower L-carnitine levels than those of the healthy controls. In addition, this study hypothesised that low L-carnitine levels in PCOS patients were associated with obesity and/or insulin resistance. Moreover, plasma L-carnitine concentrations were found to be statistically similar in PCOS patients and healthy controls, when controlled for obesity. This study implied that L-carnitine could be used as an adjunctive therapy in the management of insulin resistance or obesity in women who have PCOS. Further research might be planned to clarify the clinical effects of L-carnitine administration in PCOS patients with insulin resistance and/or obesity.

Introduction

One of the most striking findings in polycystic ovary syndrome (PCOS) is the android-type adiposity related to insulin resistance, which is characterised as visceral and abdominal subcutaneous adipose tissue accumulation. It is known that 30–60% of all PCOS patients are overweight or obese (Behboudi-Gandevani et al. 2016; Bil et al. 2016). It has been shown that hyperandrogenism and hyperinsulinaemia are improved in PCOS patients who lose 5–7% of their basal weight (Barber et al. 2016).

The etiopathogenesis of PCOS has yet to be clarified; however, insulin resistance, hyperandrogenemia and changes in gonadotropin dynamics are thought to play role in its pathophysiology. Insulin resistance is found in 25–60% of PCOS patients, and thus, insulin sensitising drugs such as metformin are usually used for the treatment of PCOS (Barber et al. 2015; Joham et al. 2016; Sirota et al. 2016).

Carnitine is a naturally occurring compound which is synthesised from the amino acids lysine and methionine. This non-essential amino acid is used for growth, development and lipid metabolism. Carnitine is obtained from either endogenous synthesis in the liver and kidneys or exogenous dietary sources such as red meat. Its biologically active form is L-carnitine. The main function of L-carnitine is the transportation of long chain fatty acids to the mitochondrial membrane for oxidation (Evans and Fornasini 2003, El-Hattab and Scaglia 2015).

Previously published studies have reported that L-carnitine increases glucose usage. Accordingly, significant improvement has been detected in insulin resistance of the patients with

type 2 diabetes mellitus (DM) after L-carnitine administration (Abdali et al. 2015; McCoin et al. 2015). Plasma total L-carnitine levels are also found to be significantly lower in PCOS patients when compared to healthy individuals (Fenkci et al. 2008).

The present study aims to determine the plasma concentrations of L-carnitine in PCOS patients with respect to obesity.

Materials and methods

This cross-sectional study was approved by the Institutional Review Board of Afyon Kocatepe University Hospital where it was conducted between May and September 2011. All the participants gave written informed consent before their enrolment into the study.

Study design and patients

Sixty women who were between 17 and 50 years old and who were diagnosed with PCOS were eligible. The 2003 Rotterdam ESHRE/ASRM diagnostic criteria were used to diagnose PCOS. A diagnosis of PCOS was made when at least two of the following three criteria were present: (1) oligo or anovulation, (2) clinical and/or biochemical hyperandrogenism findings and (3) ultrasonographic polycystic ovary appearance (The Rotterdam ESHRE/ASRM-Sponsered PCOS Workshop Group 2004).

The PCOS patients were divided into two groups according to obesity: Group 1 included 34 PCOS patients who had a

body mass index (BMI) $< 30 \, \text{kg/m}^2$. Group 2 consisted of 26 patients who had a BMI $>29.9 \, \text{kg/m}^2$. Group 3 consisted of 28 healthy women who had no menstrual irregularity, obesity (BMI $< 30 \, \text{kg/m}^2$), hirsutism and/or hyperandrogenism.

Oligo-anovulation was diagnosed clinically. Anovulation referred to an amenorrhoea of 3 months or longer without pregnancy. Oligoovulation indicated the occurrence of six menstrual cycles in a year. Hyperandrogenism was clinically diagnosed by the existence of hirsutism (modified Ferriman–Gallwey score >8) and/or acne.

The women with systemic diseases (cardiovascular disease, thyroid disease, hypertension, DM, malignancy, chronic renal disease, chronic liver disease, acute or chronic infection or rheumatologic inflammatory disease), the women with other endocrinological disorders (congenital adrenal hyperplasia, androgen secreting tumours, Cushing syndrome, thyroid disorders and prolactinoma) and the women who used oral contraceptives, anti-diabetics, anti-hypertensives, anti-hyperlipidaemics, glucocorticoids and ovulation induction drugs during the last six months were excluded.

The BMI was calculated according to the following formula:

$$\text{Body mass index } (\text{kg/m2}) = \text{Weight } (\text{kg})/\text{Height}^2 (\text{m}^2)$$

Waist circumference was measured from the umbilicus and hip circumference was measured at the level of the great trochanter.

Laboratory studies

After an 8-h-long fasting, three venous blood samples were drawn from each participant by standardised phlebotomy technique. The first blood sample was designated to measure serum glucose concentrations while the second blood sample was used to specify the concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH), thyroid-stimulating hormone (TSH), prolactin, oestradiol, total testosterone, dehydroepiandro-stenedione sulphate (DHEAS), 17-hydroxy-progesterone and insulin.

The remaining blood samples were reserved for the measurement of serum total ʟ-carnitine levels which were determined before the surgery was performed. These blood samples were centrifuged at 4000 rpm for 10 min in order to remove cellular contents. Then, the supernatants were collected and stored at $-80\,^\circ$C until the total ʟ-carnitine levels were determined. After the frozen samples were thawed, serum total ʟ-carnitine concentrations were measured by means of cytokine-specific and enzyme-linked immunosorbent assays (Cusabiotech Biotech, Wuhan, Hubei Province, China). The assays had a range of standards differing from 0.5 to 100.0 μmol/l. The inter- and intra-assay coefficients of variation were $<5\%$ and, in order to avoid inter-assay variance, samples from endometrial cancer patients and healthy controls were measured in parallel and duplicate.

The index of homeostasis model assessment-insulin resistance (HOMA-IR) was computed by the following formula:

$$\text{HOMA} - \text{IR} = [\text{Fasting plasma glucose } (\text{mg/dL})$$
$$\times \text{ Fasting plasma insulin } (\mu\text{IU/mL})]/405$$

HOMA-IR >2.7 was accepted as insulin resistance (Matthews et al. 1985).

Statistical analysis

Collected data were analysed by Statistical Package for Social Sciences version 17.0 (SPSS Inc., Chicago, IL). Continuous variables were expressed as mean ± standard deviation (range: minimum–maximum) and categorical variables were denoted as numbers or variables where appropriate. Distribution of data was evaluated by the Kolmogorov–Smirnov test. Comparisons were made by means of Student's t-test, one-way analysis of variance, Mann–Whitney U test and Kruskal–Wallis test. The correlations among the variables were detected by Pearson's correlation test. The effects of confounding variables (obesity, BMI $<30 \, \text{kg/m}^2$ and $>29.9 \, \text{kg/m}^2$ and waist-to-hip ratio, <0.85 and >0.85) on plasma ʟ-carnitine concentrations were estimated using logistic regression analysis. Two-tailed p values less than .05 were accepted to be statistically significant.

Results

Table 1 summarises the demographic characteristics of PCOS patients and control subjects. When compared to the non-obese PCOS and control groups, the obese PCOS group had significantly higher weight, BMI, modified Ferriman–Gallwey

Table 1. Demographic characteristics of the study groups.

	Non-obese PCOS ($n = 34$)	Obese PCOS ($n = 26$)	Control ($n = 28$)	p
Age (years)	23.8 ± 5.3	24.3 ± 5.4	$24,9 \pm 6,6$	.766
Height (cm)	155.0 ± 7.7	157.1 ± 5.3	159 ± 6.0	.180
Weight (kg)	59.1 ± 6.7	81.8 ± 13.1	58.5 ± 7.1	$<.001$
Body mass index (kg/m^2)	22.8 ± 2.6	33.0 ± 4.5	22.9 ± 2.9	$<.001$
Waist (cm)	71.5 ± 14.7	92.3 ± 11.2	75.2 ± 10.3	$<.001$
Hip (cm)	98.1 ± 7.2	109.1 ± 9.2	101.1 ± 8.8	$<.001$
Waist/Hip	0.74 ± 0.05	0.83 ± 0.08	0.73 ± 0.05	$<.001$
Clinical features				
Infertility	3 (8.8%)	5 (19.2%)	5 (17.9%)	.454
Hirsutism	27 (79.4%)	17 (65,4)	1 (3.6%)	$<.001$
Irregular menses	28 (82.4%)	20 (76,9)	5 (17.9%)	$<.001$
Menstrual cycle				$<.001$
Normal	9 (26.5%)	9 (34.6)	28 (100.0%)	
Oligomenorhoea	21 (61.8%)	16 (61.6%)	–	
Amenorrhoea	4 (11.7%)	1 (3.8%)	–	
Modified Ferriman–Gallwey score	15.4 ± 6.6	14.1 ± 7.8	1.7 ± 1.2	$<.001$

Table 2. Biochemical characteristics of the study groups.

	Non-obese PCOS ($n = 34$)	Obese PCOS ($n = 26$)	Control ($n = 28$)	p
Follicle-stimulating hormone (mIU/ml)	5.9 ± 1.5	5.9 ± 1.4	6.3 ± 1.3	.522
Luteinizing hormone (mIU/ml)	8.7 ± 4.8	7.2 ± 3.9	6.5 ± 3.5	.187
Thyroid-stimulating hormone (μIU/ml)	2.0 ± 1,0	1.9 ± 1.0	2.1 ± 0.9	.830
Prolactin (ng/ml)	14.2 ± 6.4	13.9 ± 4.7	16.5 ± 7.5	.369
Oestradiol (pg/ml)	49.3 ± 32.8	53.6 ± 28.3	60.8 ± 58.1	.484
Total testosterone (ng/dl)	37.9 ± 16.1	35.8 ± 1.4	31.3 ± 15.9	.238
Dehydroepiandrosterone sulphate (μg/dl)	248.1 ± 97.0	257.0 ± 129.2	209.2 ± 101.1	.228
17-Hydroxy-progesterone (ng/ml)	1.0 ± 0.7	1.1 ± 0.9	0.8 ± 0.2	.481
Homeostatic model assessment	1.9 ± 1.5	5.4 ± 1.4	2.0 ± 0.9	<.001[a,b]
Insulin resistance	5 (14.7%)	19 (73.1%)	5 (17.9%)	<.001[a,b]
L-carnitine (μmol/L)	27.5 ± 13.5	22.1 ± 13.7	43.0 ± 18.1	<.001[b,c]

[a]There is statistical significance between non-obese and obese PCOS groups.
[b]There is statistical significance between obese PCOS and control groups.
[c]There is statistical significance between non-obese PCOS and control groups..

scores waist and hip measurements, as well as waist/hip ratios ($p < .001$ for each). Hirsutism and irregular menses were significantly more prevalent in the PCOS groups than the control group ($p < .001$ for both).

Table 2 compares the biochemical characteristics of the groups. When compared to the non-obese PCOS and control groups, the obese PCOS group had significantly higher HOMA-IR indices ($p < .001$). Insulin resistance was significantly more prevalent in the obese PCOS group than the non-obese PCOS and control groups ($p < .001$ for both). When compared to the control group, plasma concentrations of L-carnitine were significantly lower in both obese and non-obese PCOS groups ($p < .001$ for both). When controlling for obesity (BMI $>30\,kg/m^2$) and increased waist-to-hip ratio (>0.85) as the confounding factors, plasma concentrations of L-carnitine were statistically similar in PCOS patients and control subjects (respectively $p = .132$ and $p = .184$).

Plasma L-carnitine concentrations correlated negatively and significantly with BMI ($r = -0.301$, $p = .020$) and HOMA-IR index ($r = -0.413$, $p = .001$) in PCOS patients.

Discussion

The clinical presentation of PCOS is highly heterogeneous, differing from menstrual irregularity alone to oligomenorrhoea complicated with metabolic syndrome. Insulin resistance is not classically addressed as a diagnostic criterion for PCOS but the presence of insulin resistance and/or obesity strongly indicates this clinical entity. A valid evidence for this indication is the relatively higher prevalence of insulin resistance and/or obesity in PCOS patients. However, insulin resistance and obesity may not necessarily accompany each other in patients with PCOS. In other words, insulin resistance or obesity may exist independently and as a result leads to distinct phenotypes (Pauli et al. 2011; Sirmans and Pate 2014).

A number of studies have focussed on the role of insulin resistance in the pathogenesis of PCOS. The findings of these studies imply that insulin resistance is closely associated with biochemical hyperandrogenemia. That is, insulin-sensitising drugs have been able to cause a significant reduction in fasting insulin levels and concurrently significant improvement in biochemical and/or clinical hyperandrogenism (Diamanti-Kandarakis et al. 2006; Diamanti-Kandarakis and Dunaif 2012).

The close relationship between insulin resistance and hyperandrogenism in PCOS patients does not have one-way direction, but, rather, follows a vicious circle. To put it another way, insulin resistance and hyperandrogenism reciprocally and continuously stimulate each other. Such an alignment of endocrinologic and metabolic alterations causes the occurrence and progressive impairment of additional morbidities which further complicate the clinical presentation of PCOS (Moghetti et al. 2013; Yuan et al. 2016).

A relationship has been established between low plasma L-carnitine concentrations and insulin resistance. Acylcarnitines are the intermediate compounds which are synthesised during fatty acid oxidation. High fructose consumption causes insulin resistance which, in turn, induces an elevation in acylcarnitines. Individuals with obesity and insulin resistance have increased concentrations of acylcarnitines as well as decreased plasma carnitine levels. Thus, it has been hypothesised that increasing carnitine availability might contribute to the improvement in glucose tolerance or insulin sensitivity (Power et al. 2007; Mynatt 2009). Additionally, it has been demonstrated that carnitine exerts positive effects on insulin resistance and L-carnitine levels correlate negatively and significantly with HOMA-IR values (Ruggenenti et al. 2009, 2010).

To the best of our knowledge, there is only one study that has researched plasma L-carnitine levels of PCOS patients. Fenkci et al. compared plasma L-carnitine concentrations of 27 non-obese PCOS patients and 30 control subjects who were matched with respect to BMI and age. Plasma L-carnitine levels of healthy patients were found to be significantly higher than those of lean PCOS patients. These results support the presence of an association between low L-carnitine levels and obesity in women with PCOS (Fenkci et al. 2008).

As for the present study, both lean and obese PCOS patients had significantly lower L-carnitine levels than those of the healthy controls. When controlling for obesity (BMI $>30\,kg/m^2$) and increased waist-to-hip ratio (>0.85) as the confounding factors, plasma concentrations of L-carnitine were statistically similar in PCOS patients and control subjects. Plasma L-carnitine concentrations correlated negatively and significantly with BMI and HOMA-IR index in PCOS patients. These findings suggest that low L-carnitine is associated with obesity in women with PCOS and this association is related with insulin resistance rather than PCOS itself. However, Fenkci et al. have proposed that the association

between L-carnitine and obesity is related with PCOS rather than insulin resistance or obesity. Such discrepancy may be attributed to the differences in the study design and the variations in phenotypes of recruited PCOS patients (Fenkci et al. 2008).

The findings of the present study should be interpreted carefully as their power is limited by several factors such as small sample size, absence of an obese control group without PCOS and lack of data about the free androgens and sex hormone-binding globulin concentrations.

Further research is required to clarify the role of L-carnitine in the pathophysiology of PCOS and subsequently consider L-carnitine as a promising pharmacological agent in the management of this endocrine disorder.

Disclosure statement

All of the authors declare that they have no competing interests.

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