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Invited editorial

Adding L-carnitine to clomiphene resistant PCOS women improves the quality of ovulation and the pregnancy rate. A randomized clinical trial

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ABSTRACT

Objective: To evaluate the effectiveness of L-carnitine on improving the ovulation and pregnancy rates as well as adverse metabolic indices in clomiphene-resistant PCOS.
Design: Single center, double blinded, superiority, randomized controlled clinical trial.
Setting: Women's Health Hospital, Assiut University.
Methods: One hundred and seventy women diagnosed with PCOS were found to be clomiphene resistant. The women were randomly allocated into two groups: Group A ($n = 85$), where patients received 250 mg clomiphene citrate from day three until day seven of the cycle plus L-carnitine (LC) 3 g daily; and Group B ($n = 85$) received 250 mg clomiphene citrate with placebo.
Outcome: Primary outcome is cumulative clinical pregnancy rate. Secondary outcomes are changes in serum glucose level and lipid profile.
Results: The combination of L-carnitine and CC significantly improve both the ovulation and the cumulative pregnancy rates in clomiphene resistant PCOS (55 (64.4%) vs. 15 (17.4%) and 44 (51.5) % vs. 5 (5.8) %). The number of stimulated follicles reaching ≥17 mm diameter was significantly more in Group A to Group B (2.2 ± 0.77 vs. 0.16 ± 0.79; $p < 0.0001$). Group A needed significantly fewer days for adequate follicular maturation, had a thicker endometrium and higher oestradiol concentration at the time of human chorionic gonadotrophin injection (10.1 ± 0.1 mm vs. 6.8 ± 0.4 mm; $p < 0.0001$). The same group had a higher mean luteal-phase serum progesterone compared with the control group (13.55 ± 0.99 vs. 10.6 ± 0.98 ng; $p < 0.0001$). A significant difference was found regarding the clinical pregnancy rates (42 (49.4%) vs. (1) 1.1% respectively p value <0.0001).
Conclusion: Adding L-carnitine when treating clomiphene-resistant PCOS patients not only improved the quality of ovulation and the pregnancy rate with an acceptable patient tolerability, but also enhanced the patient lipid profile and body mass index.

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Introduction

Repeated ovulation induction decreases the quantity of mitochondrial DNA and increases 8- hydroxydeoxyguanosine in oocytes [1,2]. The use of kinetic analysis has previously affirmed that the ovulated oocytes number declined markedly with repeated cycles of ovulation. In addition, a decrease in the gene expression of mitochondrial transcription factor A and a more incidence of oocytes with abnormally distributed mitochondria was reported [2].

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders, affecting about 6–10% of women in their reproductive age. Anovulation and hyperandrogenism are often present together with hyperinsulinaemia and insulin resistance [3]. Clomiphene citrate (CC) remains the standard drug for induction or augmentation of ovulation [4]. Conception rate in CC treated cycles is about 40% only, although the induced ovulation rates are between 80% and 85% [5,6]. It is common to start clomiphene treatment at 50 mg, and then increase the dose to 150 mg and continuing the latter dose for three consecutive cycles. If no ovulation occurred, this defines clomiphene resistance [7]. Improving the pregnancy rate in CC induction cycles has been tried repeatedly through adjuvant treatment such as *N*-acetyl cysteine [8]. For women with PCOS, treatment of insulin resistance with an insulin sensitizer such as metformin does increase the pregnancy rate [9,10].

Carnitine is a quaternary ammonium compound that can be biosynthesized from the two amino acids lysine and methionine [11]. In living cells, it helps in the transport of fatty acids from

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cytosol into the mitochondria during the breakdown of lipids in the process of generating metabolic energy. It is widely available as a nutritional supplement. Carnitine exists in two stereoisomers: L-carnitine which is the biologically active form, and D-carnitine which is the biologically inactive form [10,11].

L-Carnitine plays essential roles in energy production, oxidative stress and glucose metabolism [12]. L-carnitine can stabilize mitochondrial membranes, increase the supply of energy to the organelle, and protect the cell from apoptotic death [13]. The use of carnitine in the treatment of insulin resistance has gained attention since the role of accumulation of acyl-CoA derivatives in the development of insulin resistance was suggested [14]. Furthermore, some recent studies point towards L-carnitine insufficiency as a cause of developing insulin resistance during states of chronic metabolic stress, such as Type II diabetes and obesity, which can be reversed by carnitine [2]. Women with polycystic ovarian syndrome had lower levels of serum L-carnitine. The decrease was correlated to hyperandrogenic and hyperinsulinemia markers [15].

The study aims to assess the effectiveness of L-carnitine on the occurrence of ovulation and clinical pregnancy. Also, the presence of a favorable effect of L-carnitine on the lipogram and body mass index will be assessed.

Materials and methods

This study is a double-blind, placebo-controlled, parallel-group study conducted at Women's Health Hospital, Assiut University, Assiut, Egypt. The study was conducted between January 2010 and March 2012. Patients included were those younger than 35 years of age, presenting with primary or secondary infertility following regular intercourse for at least one year and diagnosed with PCOS with no other abnormalities. This was based on the European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine (ESHRE/ASRM) guidelines criteria (Rotterdam criteria 2003). Clomiphene resistance was diagnosed in the following manner: patients who received five CC tablets (maximum dose of 250 mg) and failed to have a follicular response after 3 cycles. The diagnosis was based on obtaining a complete history, a physical examination and a documented complete infertility work-up within the previous six months, either conducted within the setting of the hospital or at a licensed infertility management clinic.

Serum follicle-stimulating hormone (FSH), luteinizing hormone (LH) and free testosterone concentrations on day three (basal) were measured and patients with FSH values equal to or exceeding 10 IU/mL were excluded. Serum oestradiol (pg/mL) was measured by radioimmunoassay using direct double-antibody kits (Pantex, Santa Monica, CA, USA) on the day of hCG administration. Serum progesterone (ng/mL) was measured on day 8 post-human chorionic gonadotrophin (hCG) injection by a radioimmunoassay using an antibody-coated tube method (Coat-A-Count; Diagnostic Product Corporation, Los Angeles, CA, USA). Insulin was measured with Axsym insulin diagnostic division 100 (Axsym; Abbot, IL). The sensitivity of the assay was 6–24 mIU/mL. The interassay and intra-assay precision of low, middle, and high controls were 6–10 u/mL, 32–48 u/mL, and 96–144 u/mL, respectively. A serum β-hCG concentration was determined 14 days after hCG injection if menses had not yet occurred. Pregnancy was defined as an increase in the serum β-hCG of concentration more than 66% on serial determinations at least two days apart. Biochemical pregnancy was defined as falling β-hCG concentration on serial determination. Clinical pregnancy was defined as a visible intra-uterine gestational sac, with a positive fetal heart beat, when viewed by transvaginal ultrasound when the β-hCG concentration is more than 1500 IU/l.

Sample size calculation was based on the primary outcome (clinical pregnancy rate). Based on a previous study of clomiphene citrate plus *N*-acetyl cysteine versus clomiphene citrate plus placebo, pregnancy rate was 21.3% vs. 0% respectively [7]. We accept a 10% increase in the pregnancy rate. Using the online statistical calculator http://www.select-statistics.co.uk/sample-size-calculator-two-proportions, a sample size of 71 women in each arm is needed using 95% confidence level and 80% power. We included 85 patients in each arm to make up for loss to follow up.

All patients were counseled about their participation in the study. A signed informed consent was obtained. Patients had the right to refuse to participate and/or withdraw from the study at any time without being denied their regular full clinical care. Personal information and medical data collected were subject to confidentiality and were not made available to a third party. A total of 276 patients were assessed for eligibility. Participants were randomly assigned to receive either clomiphene plus L-carnitine in the capsule form (Group A) or clomiphene alone plus placebo (Group B) using previously prepared sealed envelopes with computer-generated numbers. Throughout the trial, access to the randomization code was available only to the pharmacist who manufactured the placebo and packed the envelopes and was not available to any of the treating physicians or patients. All subjects received CC from day three until day seven of the cycle. Group A received 250 mg/day CC from day three to day seven of the cycle plus oral-carnitine (3 g) supplementation from day three until the day of the first positive pregnancy test. Group B received oral CC (250 mg/day) plus placebo. The placebo capsules were specially manufactured to look identical to the L-carnitine capsules. The capsules were placed in sacks and then stored in envelopes numbered from 1 to 170. The envelopes were numbered and randomized according to computer-generated randomization tables to ensure an equal number of patients in each arm (1:1 ratio). Transvaginal follicular monitoring was performed on all patients on days seven and nine and then individualized according to response. When one leading follicle attained a diameter of 17 mm or more, 10,000 IU of hCG was given (im injection; Pregnyl, Organon, Holland). Serum LH and oestradiol concentrations were estimated on the day of the hCG injection. Timed intercourse was advised starting every other day for one week from the night of hCG administration. No luteal-phase support was given in either group. Primary outcome was pregnancy and was defined as a visible intra-uterine gestational sac, with a positive fetal heart beat, viewed during a transvaginal ultrasound (when the β-hCG concentration was more than 1500 IU/l). Fig. 1 shows a flow diagram of the patients' enrolment, allocation, follow-up and analysis.

Data was analysed according to the intention to treat principle. Data obtained was statistically analysed using SPSS version 13.0 (SPSS, Inc. Chicago, IL, USA). Chi-squared test or, if necessary, Fisher's exact test were used to compare categorical data. Continuous data are analyzed with Student's *t*-test. Data was expressed as the mean ± standard deviation (SD) for continuous variables. After primary analysis, subgroup analysis was performed to assess treatment effects on the body mass index and serum lipid profile, 50 gm for 2 h oral glucose tolerance test and glysolated hemoglobin (Hb A1c).

Results

A total of 276 patients were recruited and followed up between January 2010 and March 2012. 106 were excluded (76 did not meet the inclusion criteria and 30 refused to participate). The study was performed at Women's Health Hospital, Assiut University, Egypt. The remaining 170 patients were randomly allocated to one or the other treatment group (Group A, $n = 85$; or Group B, $n = 85$).

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Eighteen patients had lost follow up and or discontinued the treatment (8 patients in Group A and 10 patients in group B).

As shown in Table 1, no evidence of statistically significant differences in age, infertility type, infertility duration, and body mass index between the two groups was demonstrated. Basal serum FSH and LH were similar in the two groups on the day of hCG injection during the cycles in which clomiphene plus L-carnitine or clomiphene plus placebo were given.

The combination of L-carnitine and CC significantly improve both the ovulation and the pregnancy rates in clomiphene resistant PCOS (64.4% vs. 17.4% and 51.5% vs. 5.8%). The number of stimulated follicles reaching ≥17 mm diameter was significantly higher in Group A as compared to Group B (2.21 ± 0.1 vs. 0.16 ± 0.7; $p < 0.0001$). Although both groups produced a comparable number of pre-ovulatory follicles, with diameter of >17 mm, Group B patients needed significantly more days (13.55 ± 0.99 vs. 8.65 ± 0.98; $p < 0.0001$) to reach follicular maturation (Table 2). The endometrium at the time of hCG administration was significantly thicker in Group A (10.1 ± 0.1 mm vs. 6.8 ± 0.4 mm; $p < 0.0001$). Serum E2, on the day of hCG administration, was significantly higher in the L-carnitine group ($p < 0.0001$). Pregnancy occurred in 42/85 cycles in Group A (54.5%) and 5/85 cycles in Group B (5.8%) and the difference was statistically significant ($p < 0.0001$). The miscarriage rate was lower in Group A (2/85; 2.3%) than in Group B (4/85; 4.7%) ($p = 0.67$).

Compared with the baseline, the decrease of total cholesterol was significant in the L-carnitine group after 12 weeks of treatment. Furthermore in the L-carnitine group, we observed a significant decrease in triglyceride and LDL cholesterol concentrations and a significant increase in HDL cholesterol concentrations after 12 weeks of treatment compared with the baseline. The analysis of covariance showed that these differences were independent of variations in BMI and Hb A1c (Table 3). No significant change was observed in the fasting serum glucose concentrations in the L-carnitine-treated group. However, there was a significant decrease in Hb A1c of 0.6% ($p < 0.001$) after the end of treatment (Table 3).

Both L-carnitine and placebo were well tolerated in all patients. In the group treated with L-carnitine, two patients complained of nausea, two of a slight headache, and two of abdominal pain. In the

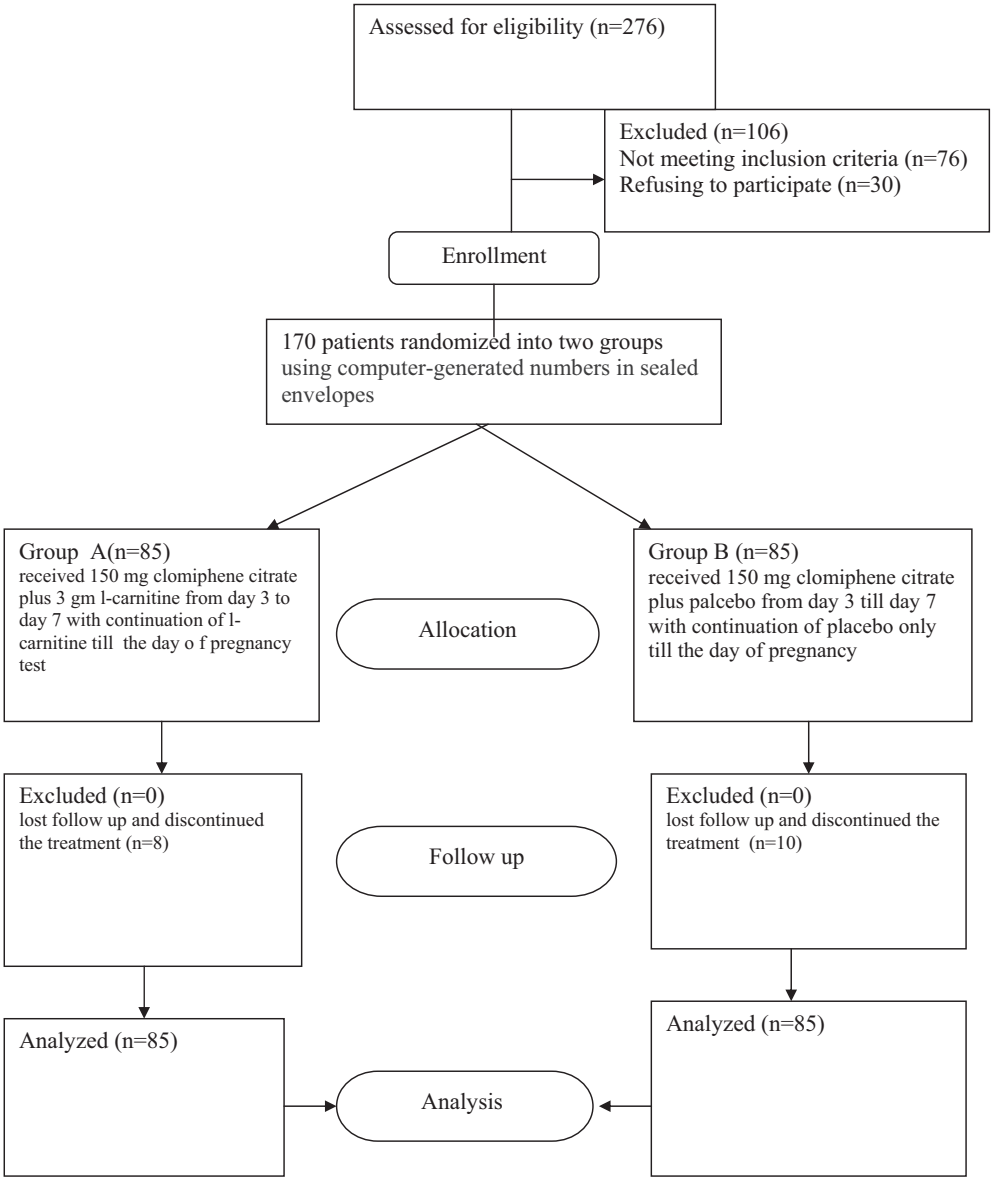

Fig. 1. A flow diagram of the patients' enrolment, allocation, follow-up and analysis.

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Table 1
Baseline characteristics of study participants.

Baseline Characteristic	CC + L-carnitine ($n = 85$)	CC ($n = 85$)	p value
Age (years)	24.6 ± 3.2	24.8 ± 2.7	0.66
Body mass index (kg/m^2)	30.6 ± 1.3	30.7 ± 1.5	0.64
Fasting insulin (U/mL)	18.8 ± 4.7	17.2 ± 4.4	0.0232
2 h post prandial 50 gm oral glucose tolerance (mm/l)	7.04 ± 1.34	7.04 ± 1.37	1
Infertility duration (months)	27.2 ± 2.6	28.7 ± 1.1	0.0001
Primary infertility (n and %)	43 (50.5)	40 (47.05)	0.68
Day three basal serum FSH (IU/mL)	4.34 ± 0.39	4.36 ± 0.41	0.74
Day three basal serum LH(IU/ML)	10.4 ± 0.45	10.5 ± 0.44	0.14
Free testosterone (ng/mL)	4.0 ± 2.4 ng/dL	4.1 ± 2.5	0.97

Values are mean ± SD unless otherwise stated; CC = clomiphene citrate; EE = ethinyl oestradiol.

placebo group, one patient complained of diarrhoea, one of nausea, and one of headache.

Comments

The problem of adequate follicular growth and good ovulation in CC-induced cycles presents a challenge to reproductive specialists [16]. There are several lines of management of clomiphene resistance in patients with PCOS including weigh reduction, insulin sensitizers such as metformin and the recently applied anti-oxidant effect of *N*-acetyl cysteine.

In the present study stimulated follicles reaching ≥17 mm diameter were found to be significantly higher in Group A ($p < 0.01$). The days required for this beneficial effect were fewer (13.55 ± 0.99 vs. 8.55 ± 0.98; $p < 0.001$) although the dose of clomiphene is lower and this may in turn explain the beneficial effects of L-carnitine on the oocyte development and maturation.

In addition, there was a significantly higher ($p < 0.001$) serum luteal phase progesterone among L-carnitine users compared with clomiphene alone. This can be attributed to one of three reasons. The first possibility is shown by the shortened follicular phase in the carnitine group (8.55 ± 0.98 days vs. 13.55 ± 0.99 days) being measured at a more advanced stage of corpus luteum development. The second explanation could be in better follicular quality based on the reversal of the deleterious effect of clomiphene on granulose cells. The third and last possibility lies in the well-known negative effects of clomiphene on oestrogen [17]. Serum oestradiol appeared to be higher on the day of hCG injection in Group A compared with Group B (277.10 ± 15.24 pg/mL vs. 245 ± 27.73 pg/mL, $p < 0.001$).

The most striking result of this treatment was the significant rise in the pregnancy rate among users compared with clomiphene-alone users (51.5% vs. 5.8%, $p < 0.001$). This can be explained in the following manner. First, the increase in progesterone receptors concentration promotes a better luteal responses to progesterone and improve pregnancy rate [18].

Second, greater endometrial thickness (10.1 ± 0.1 mm vs. 6.8 ± 0.4 mm; $p < 0.0001$). Third, in addition to its anti-oxidant effect, L-carnitine also can decrease the level of apoptosis in the presence of an apoptotic-inducer and decrease the anti-proliferative effect induced by the presence of cytokines such as TNF-α [19]. L-carnitine was reported to down-regulate cytokines such as IL-1, IL-6, and TNF-α and/or increases the clearance of these cytokines in rats implanted subcutaneously with a sarcoma tumour [20]. This may clarify the beneficial effects on the endometrial and sub-endometrial blood flow which in turn could improve the endometrial receptivity in the peri-implantation period. In the current study, sub-group analysis of the L-carnitine group demonstrates improvement of the body weight reflected in the decrease of the body mass index after twelve weeks of treatment. Furthermore, this effect was noticed on the lipid profile (25.6 ± 1.3 vs. 19.2 ± 1.6; $p < 0.001$).

To our knowledge, this is the first study of L-carnitine as adjuvant therapy for clomiphene-resistant PCOS. The positive effects of L-carnitine on the quality of ovulation and the pregnancy rate and thus, on the biochemical profile of PCOS patients appears to encourage its use in clinical practice. A need exists for a large multi-centre study to evaluate these effects and its use as a first line of induction of ovulation in PCOS.

The idea of adding L-carnitine in the follicular phase is to revert the reactive oxygen species (ROS) and act as scavenger for the harmful oxidative stress substances accumulated by previous cycles of induction of ovulation. A recent study by Kuscu and Var demonstrated up-regulated superoxide dismutase (SOD) activity in patients with PCOS compared to controls [21]. Insulin resistance and hyperglycemia are established as factors that increase oxidative stress. Fulghesu et al. evaluated the effect of *N*-acetyl-cysteine (NAC), known to replenish stores of the anti-oxidant glutathione, on insulin secretion and peripheral insulin resistance in subjects with PCOS [22]. Their results suggest that oxidative stress associated with ovulation lies in the mechanism of ovarian aging. Furthermore, L-carnitine may have therapeutic potential in patients with infertility and high incidence of aneuploidy, and may be able to suppress impaired zygote maturation usually observed in childbearing at an advanced age. Also this immune modulation may explain the lower miscarriage rate among Group A patients (2.3% vs. 4.7%; $p < 0.001$). No cytogenetic analysis of the products of conception was offered to aborting cases; hence ruling out genetically defective pregnancies was not possible.

Although L-carnitine has been advocated as a weight-loss supplement, there is no research evidence supports that. However, some studies show that oral carnitine reduces the body fat and fatigue mass with increase in the muscle mass. All of these effects

Table 2
Comparison between the clinical outcomes of the two treatment groups.

Item	CC + L-carnitine ($n = 85$)	CC ($n = 85$)	p value
Ovulation rate	55 (64.7%)	15 (17.6%)	<0.0001
Days until HCG injection	8.55 ± 0.99	13.65 ± 0.98	<0.0001
Endometrial thickness (mm)	10.66 ± 0.68	6.08 ± 0.59	<0.0001
Mean number of pre-ovulatory follicles >17 mm	2.21 ± 0.77	0.16 ± 0.79	<0.0001
Serum oestradiol on day of HCG (pg/mL)	277.10 ± 15.24	200.0 ± 27.73	<0.0001
Serum progesterone (ng/mL)	13.52 ± 0.89	10.15 ± 1.99	<0.0001
No. of pregnancies (%)	44 (51.5%)	5 (5.8%)	0.000
No. of clinical pregnancies (%)	42 (49.4%)	1 (1.1%)	0.000
No. of miscarriages (%)	2 (2.4%)	4 (4.7%)	0.67
No. of multiple pregnancies (%)	5 (5.8%)	0	

Miscarriages were defined as biochemical pregnancies and/or cases with positive b-HCG testing who aborted spontaneously before reaching the stage of clinical pregnancy and/or cases aborting before 12 weeks of pregnancy.

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Table 3
Laboratory parameters in patients treated with L-carnitine before and after 12 weeks' treatment.

Characteristics	Pre treatment	Post treatment	*p* value
LDL cholesterol (mg/dL)	151 ± 15.3	115 ± 10.2	0.000
HDL cholesterol (mg/dL)	36.3 ± 14.5	43.5 ± 13.4	0.000
Total cholesterol (mg/dL)	230 ± 12.6	190 ± 11.3	0.000
Triglycerids (mg/dL)	355.5 ± 13.7	142 ± 10.4	0.000
BMI (kg/m^2)	32.6 ± 1.3	19.2 ± 1.6	0.000
Glucose (mmol/L)	7.04 ± 1.34	6.31 ± 1.18	0.0002
Hb A1c (%)	7.3 ± 0.8	6.6 ± 0.8	0.000

Paired *t*-test All values are means ± SDs. Hb A1c, glycosylated hemoglobin percent.
LDL cholesterol
Desirable: less than 200 mg/dL (5.18 mmol/L), borderline high: 200–239 mg/dL (5.18– 6.18 mmol/L), high: 240 mg/dL (6.22 mmol/L) or higher.
Total cholesterol
Optimal: less than 100 mg/dL (2.59 mmol/L), near/above optimal: 100–129 mg/dL (2.59–3.34 mmol/L), borderline high: 130–159 mg/dL (3.37–4.12 mmol/L), high: 160–189 mg/dL (4.15–4.90 mmol/L), very high: greater than 190 mg/dL (4.90 mmol/L).
HDL cholesterol
Low level, increased risk: less than 40 mg/dL (1.0 mmol/L) for men and less than 50 mg/dL (1.3 mmol/L) for women, average level, average risk: 40–50 mg/dL (1.0–1.3 mmol/L) for men and between 50–59 mg/dl (1.3–1.5 mmol/L) for women, high level, less than average risk: 60 mg/dL (1.55 mmol/L) or higher for both men and women.
Fasting triglycerides
Desirable: less than 150 mg/dL (1.70 mmol/L), borderline high: 150–199 mg/dL (1.7–2.2 mmol/L), high: 200–499 mg/dL (2.3–5.6 mmol/L), very high: greater than 500 mg/dL (5.6 mmol/L).

may indirectly contribute to weight loss [23]. Furthermore, whereas researchers in the past century failed to prove that dietary supplementation can increase muscle carnitine content, this may have been in part due to inadequate lengths of the supplementation periods [23]. In animal study (2008) and human study (2011), researchers using L-carnitine L-tartrate supplementation for six months to assess the effects of L-carnitine on obesity, diabetes, and as an ergogenic aid, demonstrated an increase in the muscle carnitine in persons without carnitine deficiencies, and a positive impact on muscle metabolism and performance. They concluded that the supplementation of L-carnitine as an antioxidant may improve lipid profiles and exercise ability in exercise-trained rats [24,25].

Carnitine supplementation studies in humans and animals demonstrate an improvement in glucose tolerance and/or insulin sensitivity and in particular during an insulin resistant state [2,25]. Lifestyle modulation such as weight reduction and the use of insulin sensitizers can increase the clinical pregnancy rate in PCOS. This agrees with our study that after twelve weeks of treatment, weight reduction and, although no effect on fasting glucose, but reduction in the level of Hb A1c, one finds a beneficial ovarian response, measured not only in the number of mature follicles but also in the endometrial thickness and receptivity reflected in the higher clinical pregnancy rate and low miscarriage number. In our study, most patients demonstrated acceptable tolerability to the drug with very few side effects.

In conclusion, adding L-carnitine to clomiphene in the follicular phase and extending through the luteal phase in patients with clomiphene-resistant PCOS, at the given dose and duration, may be of benefit to the quality of ovulation and the clinical pregnancy rate. An improved effect on body weight and lipid metabolism further ameliorates the quality of life of these patients. Therefore, as a result of its beneficial effect and its triple effects on oxidative stress, lipid metabolism and glucose metabolism, this treatment may be added as a first line in the management of PCOS.

Conflicts of interests

The authors have no conflict to disclose.

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