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Improved insulin sensitivity and reproductive profile in overweight/obese PCOS patients undergoing integrative treatment with carnitines, L-arginine, L-cysteine and myo-inositol

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ABSTRACT

Objective: To evaluate the effects of a combination of carnitines, L-arginine, L-cysteine and myo-inositol on metabolic and reproductive parameters in PCOS overweight/obese patients.

Methods: This was a retrospective study analyzing information of a group of PCOS ($n=25$) overweight/obesity patients, not requiring hormonal treatment, selected from the database of the ambulatory clinic of the Gynecological Endocrinology Center at the University of Modena and Reggio Emilia, Modena, Italy. The hormonal profile, routine exams and insulin and C-peptide response to oral glucose tolerance test (OGTT) were evaluated before and after 12 weeks of a daily oral complementary treatment with L-carnitine (500 mg), acetyl-L-carnitine (250 mg), L-arginine (500 mg), L-cysteine (100 mg) and myo-inositol (1 gr). The hepatic insulin extraction index was also calculated.

Results: The mix of complementary substances significantly improved metabolic parameters, homeostatic model assessment for insulin resistance index values and gonadotropin plasma levels. Glucose, C-peptide and insulin response to OGTT was significantly reduced as well as the hepatic insulin extraction index.

Conclusion: The administration of a combination of carnitines, L-arginine, L-cysteine and myoinositol improved gonadotropin plasma levels and insulin sensitivity in overweight/obese PCOS patients and restored hepatic clearance of insulin as demonstrated by the decreased hepatic insulin extraction index.

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Introduction

Reproductive impairment is typically occurring in a high percentage of women diagnosed with the polycystic ovary syndrome (PCOS) which covers up to 20–25% of women in reproductive age [1]. It is now well agreed that the diagnosis of PCOS relies on the presence of 2 out of 3 of the criteria established at the consensus meeting in Rotterdam [2]. However, during these last years the dysregulation of metabolic aspects observed in PCOS patients have raised interest. In fact, very frequently PCOS patients display overweight up to obesity together with compensatory hyperinsulinemia. Such hyperinsulinism is tightly related to the amount of fat tissue and it is considered not only a relevant trigger for the hyperandrogenic condition, being insulin a co-gonadotropin at the ovarian level, but also a very frequent predisposing factor for the onset of the metabolic syndrome (METS) in PCOS women [3]. For this reason, in recent years it has been proposed that the term PCOS be changed to 'metabolic reproductive syndrome' in consideration of the constellation of cardiometabolic risk factors [4,5].

Being compensatory hyperinsulinemia frequent in up to 50–70% of obese PCOS patients and in 15–30% of normal weight PCOS patients [3], we evaluated it since for PCOS patients it represents a putative trigger not only for the development of the METS [6,7], but also for nonalcoholic fatty liver disease (NAFLD) and/or liver fibrosis, both very frequent in PCOS patients as compared to healthy women [8]. During the last decades several therapeutic strategies have been proposed, using both classical insulin sensitizing drugs (i.e. metformin) [9] and integrative approaches, using specific natural compounds discovered as integrated into the cellular pathways driving the metabolic activities. Inositols (both myo-inositol and D-chiro inositol) [10,11], alpha lipoic acid [12], carnitines [13], N-acetyl cysteine [14], L-arginine [15] or various combination of these [12,16–20] have shown to be beneficial to face the impairments that PCOS patients display [21]. In fact, each one or in combination have been reported to reduce insulin resistance acting on peripheral tissues, thus improving insulin sensitivity [10,12,16,17] and reproductive hormonal parameters [10,12,13,15]. In addition, some of these integrative compounds have been demonstrated to act as anti-oxidants, thus sustaining the endogenous anti-oxidative system of which the most important representative is glutathione. Among the many integrative compounds, carnitines, L-arginine and L-cysteine have recently demonstrated to be effective not only in patients with functional hypothalamic amenorrhea [22] but also in PCOS patients [16]. Such ability to improve metabolic profiles in

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completely different settings that display reproductive impairments, supports the hypothesis that metabolism and the integrative approach that improves it, plays a crucial role in modulating and driving reproductive functional ability.

On such basis, we aimed to evaluate the effects of a combination of carnitines, L-arginine, L-cysteine with the addition of myo-inositol on both reproductive and metabolic parameters in a group of overweight/obese PCOS patients.

Methods

Study design and participants

From the database of PCOS patients attending the ambulatory clinic of the Gynecological Endocrinology Center at the University of Modena and Reggio Emilia, Modena, Italy between January 2022 and April 2024 a group of overweight/obese PCOS patients ($n = 25$) were selected that included those not requiring hormonal treatment. On such basis, we selected the patients that received an integrative approach with an oral combination of L-carnitine (500 mg), acetyl-L-carnitine (250 mg), L-arginine (500 mg), L-cysteine (100 mg) and myo-inositol (1 gr) and that had completed the baseline screening and the first clinical check after 12 weeks of treatment. All PCOS patients were selected in accordance to the diagnostic criteria established by the American Society for Reproductive Medicine and the European Society for Human Reproduction and Embryology [2], and with at least two of the following criteria: (a) oligomenorrhea with inter-menstrual intervals longer than 45 days, (b) clinical (acne, hirsutism) or biochemical signs of hyperandrogenism, (c) presence of micro-polycystic ovaries (i.e. ≥ 20 follicles per ovary and/or an ovarian volume ≥ 10 ml on either ovary) detected by a ultrasound transducer with a frequency bandwidth that includes 8 MHz [23].

In addition, patients had to fulfill the following criteria: (a) absence of enzymatic adrenal deficiency and/or other endocrine disease, including diabetes, (b) normal prolactin levels (range 5–25 ng/mL), (c) no hormonal treatment during a period of at least 6 months prior to the study, and (d) increased body mass index (BMI). None of the subjects was taking medications and/or steroids, or other drugs (i.e. contraceptives or metformin) within the 3 months prior to the initial evaluation.

As a routine procedure, our center screens PCOS subjects before and after at least 3 months (12 weeks) of treatment as follows: on day 3–6 of the menstrual cycle and on day 3–6 of the first bleeding occurring after the 12th week of treatment. The following parameters were evaluated: luteinizing hormone (LH), follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), estradiol, progesterone, prolactin, androstenedione, 17-hydroxyprogesterone, dehydroepiandrosterone sulfate, insulin, glutamic aspartate amino transferase and alanine amino transferase. Total cholesterol, HDL-C, LDL-C, and triglyceride plasma levels were also evaluated. The Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) index was computed to estimate sensitivity to insulin [24].

In addition to the hormonal and metabolic baseline profile, an oral glucose tolerance test (OGTT) was performed at fasting baseline and at 30, 60, 90, 120, 180 and 240 min after the oral intake of 75 g of glucose. This was performed before and after the 12 weeks of the integrative treatment to evaluate blood glucose, insulin and C-peptide response and to assess the eventual hyperinsulinemic response which had to be above 50 μIU/mL within 90 min of glucose load [25].

Informed consent is always obtained from all individual referring to the out-patient ambulatory clinic of the Gynecological Endocrinology Center as a standard procedure of the University of Modena and Reggio Emilia, Modena, Italy, before proceeding to any diagnostic investigation. The study was approved by the local Ethical Committee as a retrospective observational study.

Blood hormonal and metabolic measurements

All samples from each subject (baseline and after treatment) were run in the same assay in order to minimize errors. Plasma LH and FSH concentrations were determined using an immunofluorometric assay, as previously described [26] with high sensitivity, expressed as the minimal detectable dose of 0.1 mIU/mL. Intra-assay and inter-assay coefficients of variation were 4.0% and 6.0%, respectively.

Plasma prolactin, estradiol, progesterone, androstenedione, TSH, 17-hydroxyprogesterone, DHEAS, insulin as well as glutamic aspartate amino transferase, alanine amino transferase, glucose, triglyceride, total cholesterol, HDL-C, and LDL-C levels were determined through standard routine procedure by the Modena Hospital Central Laboratory. Based on two quality control samples, the average within- and between-assay coefficients of variation of the assays were 4.2% and 10.4%.

Statistical analysis

Statistical analysis was performed with QuickStatCalculations (https://www.socscistatistics.com/). Data are presented as mean ± standard error of the mean (SEM). Analysis of variance was performed using *one-way ANOVA* to test for statistically significant differences between the groups (before and after the treatment) and then Student's *T* test was applied for paired and unpaired data, as appropriate.

To estimate insulin sensitivity the HOMA-IR index was computed [24] and calculated as: [fasting insulin mIU/L × fasting glucose mmol/L]/22.5 [24]. The cutoff value used to define insulin resistance was 2.71, as previously reported [24,25]. Additionally, the hepatic insulin extraction index was calculated as the *ratio* between insulin and C-peptide plasma concentrations (insulin/C-peptide), as previously reported [27,28]. This index was determined upon all OGTT samplings. The hepatic insulin extraction index reflects insulin kinetics as a balance between pancreatic insulin synthesis and its hepatic clearance. C-peptide almost exclusively reflects pancreatic synthesis because hepatic C-peptide clearance is minimal [29–31].

The area under the curve (AUC) of insulin and AUC of hepatic insulin extraction under the OGTT glucose load was computed using the trapezoid formula subtracting the baseline values.

Results

The hormonal and metabolic parameters of the group of PCOS patients under consideration are reported in Table 1. LH and FSH plasma levels were significantly reduced after the treatment interval as well as insulin, glucose, total cholesterol and LDL-C concentrations. HOMA-IR index improved significantly as well as the hepatic insulin extraction index (Table 1).

Response to OGTT significantly improved after treatment. In fact, the glucose response resulted reduced in 5 blood samples over the 8 ones of the OGTT (Figure 1). Similarly, C-peptide

Table 1. Hormonal and metabolic parameters of PCOS patients under study ($n = 25$) before and after 4 months of integrative treatment.

	LH mIU/mL	FSH mIU/mL	TSH µIU/mL	E2 pg/mL	P ng/mL	PRL ng/mL	A ng/mL	17-OHP ng/mL	Glucose mg/dL	Insulin µIU/mL	C-peptide mg/dL	Total cholest mg/dL	HDL-C mg/dL	LDL-C mg/dL	TG mg/dL	AST IU/L	ALT IU/L	DHEAS ng/mL	HOMA-IR index	LH/FSH	BMI	HIE index
Baseline																						
Mean ±	9.1 ±	6.3 ±	2.6 ±	53.0 ±	1.0 ±	17.2 ±	2.1 ±	1.3 ±	95.2 ±	19.2 ±	2.7 ±	191.5 ±	52.7 ±	121.2±	107.7 ±	24.8 ±	25.3 ±	2.0 ±	4.6 ±	1.4 ±	34.8 ±	6.7 ±
SEM	0.76	0.43	0.25	8.38	0.27	2.54	0.20	0.20	2.94	2.71	0.3	6.44	2.78	6.86	12.19	1.57	3.37	0.18	0.78	0.09	1.87	0.59
After treatment																						
Mean ±	7.4 ±	5.1 ±	2.9 ±	68.6 ±	2.2 ±	13.3 ±	2.07 ±	1.6 ±	85.7 ±	13.0 ±	2.53 ±	171.9 ±	51.8 ±	95.4 ±	101.6 ±	24.1 ±	20.1 ±	2.0 ±	2.9 ±	1.5 ±	34.9 ±	5.6 ±
SEM	0.74	0.38	0.41	13.50	1.13	2.71	0.25	0.32	4.50	2.15	0.26	8.28	3.59	6.69	10.18	2.31	2.81	0.34	0.63	0.11	4.40	0.67
p value	0.03	0.01							0.02	0.05		0.03		0.05					0.04			0.02

Data are expressed as mean ± standard error of the mean (SEM); LH, luteinizing hormone; FSH, follicle stimulating hormone; TSH, thyroid stimulating hormone; E2, estradiol; P, progesterone; PRL: prolactin; A, androstenedione; 17-OHP, 17 hydroxyprogesterone; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TG, triglyceride; AST, aspartate amino transferase; ALT, alanine amino transferase; HOMA-IR, homeostatic model assessment for insulin resistance; BMI, body mass index; HIE, hepatic insulin extraction.

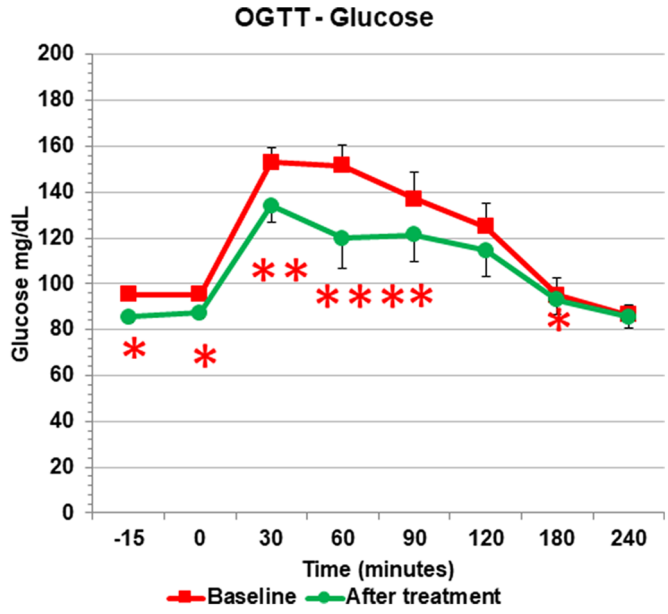

Figure 1. Glucose response to glucose load before (red) and after (green) the integrative treatment interval. *$p < 0.05$; **$p < 0.01$; ***$p < 0.004$.

and insulin response to OGTT were significantly reduced after the treatment interval, showing some peculiar differences. While, C-peptide showed a significant decrease only 60 min after the glucose load (Figure 2, Panel A), conversely, insulin showed a significant decrease at baseline conditions and also 60 and 90 min after the glucose load (Figure 2, Panel B) so that the AUC of insulin response to OGTT resulted significantly reduced (Figure 3).

To better understand the changes in the dynamics of the compensatory hyperinsulinemia observed after the integrative treatment, we computed the hepatic insulin extraction index [7] at each OGTT sampling time. Thus, the ratio between insulin and C-peptide concentrations was computed for each sampling time. Since the hepatic insulin extraction index is specific to hepatic insulin clearance/extraction by the liver, any changes observed might allow specific clinical considerations. Considering the data for the whole set of PCOS patients, the hepatic insulin extraction index significantly decreased after 12 weeks of integrative treatment (Figure 4, Panel A) so that the computed AUC also resulted significantly reduced after treatment (Figure 4, Panel B).

Discussion

The present study demonstrates that the combination of carnitines, L-cysteine, L-arginine with the addition of myo-inositol significantly improves both metabolic and reproductive parameters in overweight/obese patients with PCOS and supports the hypothesis that part of the compensatory hyperinsulinemia observed in these PCOS patients is related to the impaired hepatic clearance of insulin. Our data support previous findings in regards to the role of the synergic action of the combination of different kinds of integrative compounds in improving insulin sensitivity and metabolic parameters in PCOS patients [16]. Several studies have reported that inositols (both myo-inositol and D-chiro inositol) [10,11], alpha lipoic acid [12,32], carnitines [13], N-acetyl cysteine [14], L-arginine [15] or various combinations of these [10,12,13,15–20] were beneficial to face the

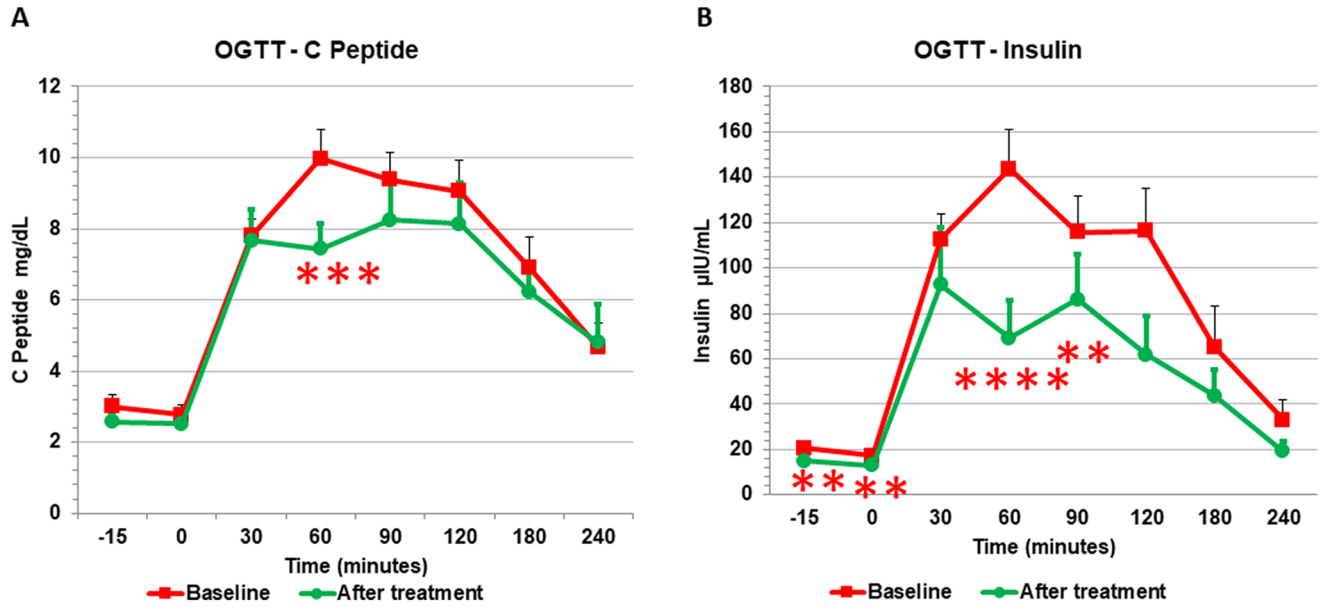

Figure 2. C-peptide (panel A) and insulin (panel B) response to glucose load before (red) and after (green) the integrative treatment interval. $^{**}p < 0.01$; $^{***}p < 0.007$; $^{****}p < 0.002$.

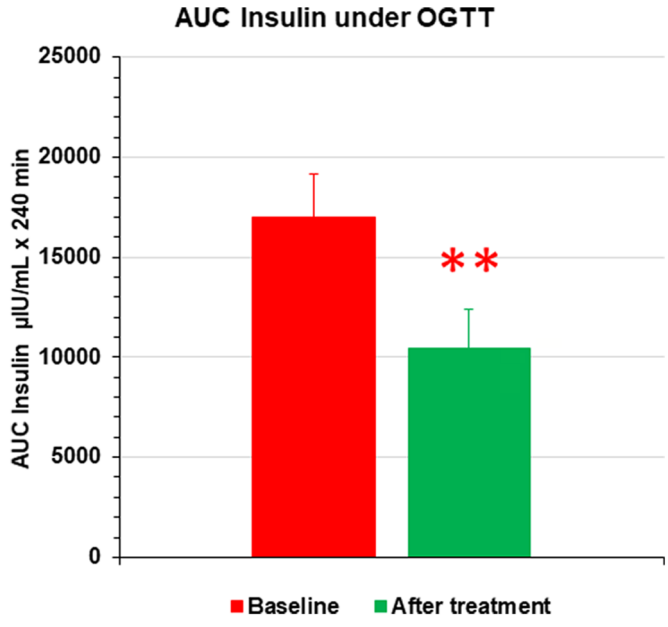

Figure 3. Area under the curve (AUC) of insulin response to glucose load before (red) and after (green) the treatment interval. $^{**}p < 0.008$.

impairments that PCOS patients display [21], improving insulin sensitivity [10,12,13,15] and reproductive hormonal parameters [10,12,16,17].

The combination of carnitines with cysteine and L-arginine previously demonstrated efficacy both in patients with PCOS [16] and those with functional hypothalamic amenorrhea [22]. Though these clinical situations are clearly different, both are characterized by a metabolic impairment that integrative combination treatment was able to improve, acting mainly on the metabolic aspects of both conditions. In this sense, metabolic aspects are essential in driving the reproductive function in humans [33]. Our present data confirm the ability of the used combined treatment to positively act on the metabolic profile of a group of overweight/obese PCOS patients. It is important to mention that this is first report in which the combination adds myo-inositol aiding in the improvement of the reproductive parameters [16]. Our group has demonstrated that the combination of carnitines with cysteine and L-arginine was able to reduce insulin resistance thus improving insulin sensitivity but with no changes for gonadotropins [16]. Contrary to this, the present study, perhaps owing to the addition of myo-inositol to the mix of complementary compounds, seems to confirms a positive effect over the metabolic parameters and insulin resistance, with a significant reduction of LH and FSH plasma levels after the treatment interval.

Interestingly, after the treatment interval not only insulin but also glucose plasma levels were significantly reduced. Such decrease was also evident in terms of glucose response under OGTT. Since, myo-inositol acts as an insulin sensitizer [33], such positive effect has to be ascribed to the presence of myo-inositol as the additional compound in the used combination. Moreover, myo-inositol's effect on insulin sensitivity and the reduction of insulin plasma, were both similar to that induced by carnitine, cysteine and L-arginine.

The fact that the addition of myo-inositol to the integrative mix induced a decrease of LH plasma levels suggests that myo-inositol effectively aided at lowering insulin and this probably enhanced a lesser insulin-induced gonadotropin release. Such effect might be ascribed to the fact that insulin plays a stimulatory role on kisspeptin-neurokinin-β-dynorphin neurons that, through kisspeptin secretion, activates GnRH release from GnRH-secreting neurons within the hypothalamus. In fact, it is well known that in humans there is a positive correlation between insulinemia and LH release and any reduction of insulin plasma levels is accompanied by a reduction of LH [3,34,35].

Of interest is the fact that after the treatment interval insulin plasma levels were significantly reduced both before as well as after the glucose load. In addition, after the treatment interval, C-peptide response was reduced but not as much as for insulin that showed a greater reduction. Such kind of distinct different effect has also been observed when treating PCOS patients with alpha lipoic acid [32]. Considering that C-peptide and insulin derive from pro-insulin cleavage within Langerhans cells of the pancreatic islets and that such cleavage generates one insulin

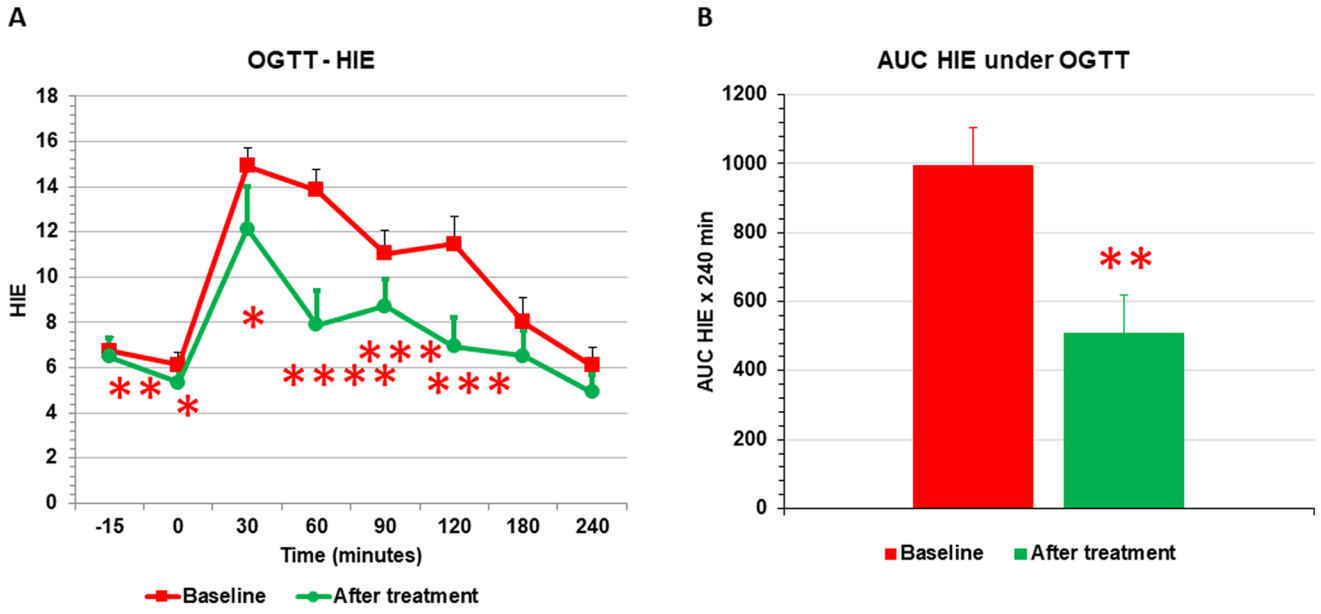

Figure 4. Hepatic insulin extraction (HIE) index throughout the oral glucose tolerance test (OGTT). After the treatment interval, HIE index (panel A) and the AUC of HIE (panel B) decreased (green) in comparison to baseline (red). *$p < 0.05$; **$p < 0.01$; ***$p < 0.0001$.

molecule and one C-peptide molecule, it comes clear that the ratio between insulin and C-peptide plasma concentrations, should be 1 [31]. Since 50% of the insulin clearance is performed by the liver and almost no clearance affects C-peptide, the ratio between insulin and C-peptide plasma levels, i.e. the hepatic insulin extraction index, reflects the liver ability to degrade insulin, since C-peptide degradation is minimal [31]. Recently, the hepatic insulin extraction index has been calculated in obese PCOS subjects, demonstrating to be tightly linked to insulin resistance [29,30]. Briefly, the hepatic insulin extraction index is the ratio between insulin and C-peptide plasma levels and reflects the balance between the kinetics of synthesis and the clearance of the two peptides. This index is usually calculated after overnight fasting [29], and has been reported to increase with the increase of insulin resistance in muscle and adipose tissues and also when there is a reduced liver ability to clear insulin [29,35]. Liver degrades insulin through the action of a specific enzyme, i.e. the insulin degrading enzyme [28]. Since the hepatic insulin extraction index reflects insulin kinetics as a balance between pancreatic insulin synthesis and its hepatic clearance, C-peptide almost exclusively reflects pancreatic synthesis because hepatic C-peptide clearance is minimal [29–31]. On such basis the index could be considered an indirect index of liver efficiency.

According to the present data, the computation of the index throughout the OGTT discloses the fact that a great part of the insulinemia observed in our PCOS patients before undergoing the integrative treatment was due to a reduced hepatic clearance of insulin rather than to an increased pancreatic production. Our data agrees with a previous study [16], confirming that such clearance resulted greatly improved by the integrative approach with carnitines, L-arginine and cysteine that restores the ability to express the insulin degrading enzyme at the hepatocyte level. The present study demonstrates such improvement throughout the OGTT, since there was a reduction of the AUC of both insulin and the hepatic insulin extraction index. Reasonably, myo-inositol seems not to be involved in such effect since no impact on liver or on transaminase plasma levels have been observed in previous studies [34], yet certainly myo-inositol participated in improving peripheral sensitivity to insulin thus inducing an additional lowering signal for pancreatic insulin secretion.

Our data confirm what has previously been proposed [16]: the combination of the used complementary compounds improved both hepatic insulin degradation and decreased insulin synthesis at the pancreatic level. Each of the complementary substances we administered have the ability, singly, to improve insulin sensitivity, reducing insulin resistance in PCOS patients [14,15,36]. It is well known that carnitines have a consistent positive effect on the Krebs cycle modulating the beta oxidative processes [37] and that the administration of carnitines in our PCOS patients improved the low circulating levels of carnitines observed in patients with PCOS [38], acting positively on cellular function at every level, both centrally (i.e. central nervous system) and in peripheral organs (i.e. skeletal muscles, fat tissue, liver, etc.). It could be inferred that L-arginine and L-cysteine administration increased the plasma levels of both these compounds observed to be low in PCOS patients [39] and both acted synergistically on this positive modulation of carnitines. In addition, both L-arginine and N-acetyl cysteine donate thiol groups and reactivate oxidative glutathione, thus also improve nitic oxide synthesis [40]. This mechanism has been demonstrated to be a protective action on endothelial cells as well as at ovarian level, and it has also been reported to drive the improvement of insulin sensitivity in PCOS patients [41,42], thus avoiding the triggering of insulin resistance and hypertensive predisposition [41–43]. The interesting fact is that, when combined, they showed a synergic positive effect at lower dosages.

In conclusion, the administration of the combination of carnitines, L-arginine, L-cysteine, and myo-inositol improved gonadotropin plasma levels and insulin sensitivity in overweight/obese PCOS patients, supporting the role of the synergistic combination at improving both the metabolic and reproductive impairments in PCOS patients.

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Author's contributions

Alessandro D. Genazzani was involved in study conception and design; Christian Battipaglia, Martina Foschi, Elisa Semprini, Claudia Aio, Eleonora Spelta, Anna Kostrzak, Maria Laura Rusce, and Anna Szeliga were involved in data collection. Alessandro D. Genazzani and Blazej Meczekalski performed drafting and writing of the manuscript. All authors were involved in critically revising the manuscript for its intellectual content, and the final approval of the manuscript was performed by all authors.

Disclosure statement

The authors declare having no conflicts of interest.

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Data availability statement

All required information regarding the study protocol and collected data will be made available upon request to researchers who provide a methodologically sound proposal. Only the analysis required to achieve the aims in the approved proposal will be permitted. Proposals should be directed to algen@unimo.it

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