Modulatory role of D-chiro-inositol and alpha lipoic acid combination on hormonal and metabolic parameters of overweight/obese PCOS patients

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ABSTRACT

Context: Polycystic ovary syndrome (PCOS) is a frequent disease characterized by several endocrine impairments and frequent metabolic abnormality, i.e. compensatory hyperinsulinemia.

Aims: To evaluate the improvements induced by a daily treatment with a combination of d-chiro-inositol (DCI) (500 mg) and alpha-lipoic acid (ALA) (300 mg) for 12 weeks.

Setting: retrospective study

Design: Thirty overweight/obese patients were evaluated. The presence/absence of first-degree diabetic relatives was ascertained. Patients were administered DCI (500mg/day) and ALA (300 mg/day) per os for at least 12 weeks. Only patients completing 12 weeks of treatment (n=30) were included in the study. Patients were evaluated before and after the treatment through measurement of plasma levels of LH (Luteinizing Hormone), FSH (Follicle Stimulating Hormone), estradiol, progesterone, androstenedione, testosterone, insulin, glutamic oxaloacetic transaminase (GOT), and glutamic pyruvic transaminase (GPT). They also underwent an oral glucose tolerance test (OGTT) to evaluate glucose, insulin and c-peptide responses.

Results: The combination treatment improved hormonal and metabolic parameters, as well as insulin and c-peptide responses to OGTT and the HOMA index. On subdividing the patients by presence/absence of familial diabetes, DCI+ALA was found to be more effective, both on metabolic and on hormonal parameters, in PCOS subjects with familial diabetes. PCOS patients with familial diabetes had higher baseline GOT and GPT levels than those with no familial diabetes and the combination treatment significantly reduced these levels.

Conclusions DCI+ALA proved to be an efficient combination that improved insulin sensitivity and hormonal and metabolic profiles in overweight/obese PCOS patients, especially those with familial diabetes, in whom it reduced the GOT and GPT levels. This latter effect might reduce the risk of non-alcoholic fatty liver disease (NAFLD), typical of PCOS patients.

KEYWORDS

PCOS, insulin resistance, NAFLD, anovulation, d-chiro inositol, alpha lipoic acid.

Introduction

Polycystic ovary syndrome (PCOS) is a frequent endocrine disease affecting 4-25% of women of reproductive age [1, 2]. The diagnostic criteria were established at the American Society for Reproductive Medicine and European Society for Human Reproduction and Embryology consensus meeting in Rotterdam [3]. A diagnosis of PCOS requires the presence of at least two of the following criteria: [4] chronic anovulation disorder (oligo or anovulation leading to amenorrhea); [5] clinical (acne, hirsutism) or biochemical signs of hyperandrogenism; and [6] the presence of micro-polycystic ovaries at ultrasound or the presence of 12 or more follicles with a diameter of 2–9 mm in each ovary, and/or increased ovarian volume (> 10 ml) [7].

In the last decade the dysmetabolic state of insulin resistance (IR) and its correlate, compensatory hyperinsulinemia, have been considered important additional aspects [8, 9]. Both are due to a deficiency of a D-chiro-inositol (DCI)-containing phosphoglycan that mediates the action of insulin [10]. Inositol improves insulin sensitivity because it works as a second messenger that may achieve an insulin-like effect on metabolic enzymes [11]. However, the presence of familial predisposition to diabetes in PCOS patients is an important consideration, since it predisposes to lower endogenous conversion of myo-inositol (MYO) to DCI as a result of reduced expression/function of the epimerase enzyme [12]. The use of both these types of inositol as a combination treatment improves insulin sensitivity [11, 12] in hyperinsulinemic PCOS patients and restores more appropriate

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metabolic control of glucose and better reproductive functions [12]. However, the use of DCI seems to be more appropriate in PCOS patients who have at least one first-degree relative affected by type I or II diabetes [12, 13]. Interestingly, PCOS women have increased oxidative stress, and this seems to contribute to the IR state [14]. In fact, increased oxidant status is related to central obesity, age, blood pressure, serum glucose, insulin and triglyceride levels, and also to IR [9, 15]. Alpha lipoic acid (ALA) is a potent antioxidant, and controlled-release ALA has been reported to improve glucose control in type II diabetes patients [14], and to improve insulin sensitivity and metabolic disorders in women with PCOS [16]. In addition, a combination of MYO and ALA can be used in insulin-resistant PCOS patients to improve their insulin sensitivity [7] and metabolic and reproductive profiles. The aim of our study was to evaluate the effects of a combination of DCI and ALA on both metabolic and hormonal parameters in a group of obese patients with PCOS.

Materials & Methods

Subjects

Among the many patients seen between January 2015 and December 2017 and recorded in the outpatients' database of our Gynecological Endocrinology Center, a total of 30 overweight/obese patients [22.5 ± 1.7 years, mean ± standard error of the mean (SEM)] was selected. All these patients required treatment for their PCOS condition (n = 30), but they were not willing to have any hormonal therapy. Informed consent was obtained from all individual participants as a standard procedure of the University of Modena and Reggio Emilia, Italy.These patients were selected according to the criteria established by the American Society for Reproductive Medicine and the European Society for Human Reproduction and Embryology for diagnosing the presence of PCOS [3], and at least two of the following criteria had to be present: (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 at ultrasound. In addition, patients had to fulfil the following criteria: (d) absence of enzymatic adrenal deficiency and/or other endocrine disease, including diabetes, (e) normal prolactin (PRL) levels (range 5–25 ng/ml), (f) no hormonal treatment during a period of at least 6 months prior to the study, (g) body mass index above 26. None of the subjects enrolled had taken medications and/or steroids, oral contraceptives or metformin within the 3 months prior to the evaluation. All the patients, at the first consultation, were interviewed to establish whether or not they had one or more first-degree relative (parents and/or grandparents) with diabetes. The anamnestic investigation revealed that 18 of the 30 patients (60%) reported first-degree diabetic relatives. All these patients were selected from the database because they had been taking a preparation combining DCI (500 mg) and ALA (300 mg) every morning at around 10 a.m. for at least 3 months (12 weeks). No lifestyle or dietary changes were required of the patients and all were studied, the first time, on day 3–6 of the menstrual cycle, if present. The post-treatment follow-up was performed after at least 12 weeks of treatment, plus a few days if necessary, so that patients were again evaluated on day 3–6 of the menstrual cycle (the first occurring after the treatment). All patients were evaluated for luteinizing hormone (LH), follicle stimulating hormone (FSH), estradiol (E2), progesterone (P), androstenedione (A), testosterone (T), insulin, glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT). HOMA index was computed to estimate sensitivity to insulin [4]. An oral glucose tolerance test (OGTT), for insulin and glucose determinations, was performed sampling before and 30, 60, 90, 120, 180 and 240 min after the oral assumption of 75 g of glucose, before and after the 12 weeks of combination treatment. A hyperinsulinemic response is recognized when insulin plasma levels are above 50 μU/ml within 90 min of glucose load [5]. The mean treatment duration was 97.5 ± 4 days [mean ± standard error of the mean (SEM)], the range being 92–113 days.

Assay

All samples from each subject were assayed in duplicate in the same assay. Plasma LH and FSH concentrations were determined using a previously described immunofluorometric assay [6, 17]. The sensitivity of the assay, expressed as the minimal detectable dose, was 0.1 IU/ml. The cross-reactivities with free and ß-subunits of LH, FSH and thyroid stimulating hormone (TSH) were less than 2% [4]. Intra-assay and inter-assay coefficients of variation were 4.3% and 6.5%, respectively. Plasma E2, A, cortisol and T were determined by radioimmunoassay (Radim, Pomezia, Rome, Italy), as previously described [18]. Based on two quality control samples, the average within- and between-assay coefficients of variation were 3.5% and 8.4%.

Plasma insulin and c-peptide concentrations were determined using an immunoradiometric assay (Biosource Europa S.A., Nivelles, Belgium). Based on two quality control samples, the average within- and between-assay coefficients of variation were 4.0% and 10.2%.

Statistical analysis

After analysis of variance (one-way ANOVA), data were tested for statistically significant differences between the groups (before and after the treatment) by means of Student's t-test for paired and unpaired data, as appropriate. The differences in insulin and c-peptide responses to OGTT were computed as maximal responses (ΔMax). ΔMax was computed as the difference between the maximal hormonal response and the hormonal concentration before the stimulation (time 0). The HOMA index was computed to estimate sensitivity to insulin [4] since it is considered the main index of the metabolic syndrome and a common link between the coexisting abnormalities; it can be calculated by homeostasis model assessment of IR (HOMA-IR) as (fasting insulin mU/l) × (fasting glucose mmol/l)/22.5 [4]. The cutoff value we used is 2.71 as previously stated [4, 5]. Data are expressed as mean ± SEM.

Results

The patients' hormonal and metabolic parameters are reported in Table 1. The administration of DCI plus ALA significantly changed LH, A, insulin and LDL plasma levels. Also, BMI and the HOMA index decreased significantly (Table 1).

As regards the OGTT, the maximal insulin and c-peptide responses (ΔMax) to the glucose load decreased significantly in the whole group of PCOS patients (Fig. 1), thus indicating the positive effects of the combination treatment. With regard to the presence or absence of familial diabetes (Table 2), the group with familial diabetes showed improved plasma LH, A and insulin levels and significantly reduced triglycerides, total cholesterol, LDL, GOT and GPT. Patients with no familial diabetes showed improvements only in plasma LH, insulin and A levels, as well as in the HOMA index (Table 2), while no changes in GOT and GPT or in the lipid profile were observed.

This subdivision of the patients revealed that in baseline conditions PCOS patients with familial diabetes showed higher GOT and GPT levels and a higher HOMA index than the other group, while insulin plasma levels were higher but without the difference reaching statistical significance (Table 2). After the treatment, GOT and GPT plasma levels decreased in PCOS with familial diabetes and became no different from those of patients without familial diabetes (Table 2).

As regards the OGTT results, different responses to glucose load were observed when considering the two subgroups of PCOS patients. Those with familial diabetes showed a significant reduction of insulin (Fig. 2 panel A) and c-peptide ΔMax (Fig. 2 panel B), greater than what was observed in PCOS patients without familial diabetes (Fig. 2 panels C and D). Moreover, the insulin ΔMax of PCOS patients with familial diabetes in baseline conditions was greater than in the other group (Fig. 1 panel A and C), similarly to the c-peptide ΔMax (Fig. 1 panels B and D). Though PCOS patients with no familial diabetes had a reduction of insulin ΔMax (Fig. 1 panel C), no changes in c-peptide ΔMax were observed (Fig. 2 panel C).

Figure 1 Maximal insulin (left) and c-peptide responses (right) (Δmax) to OGTT in all PCOS patients under study. ** p< 0.005.

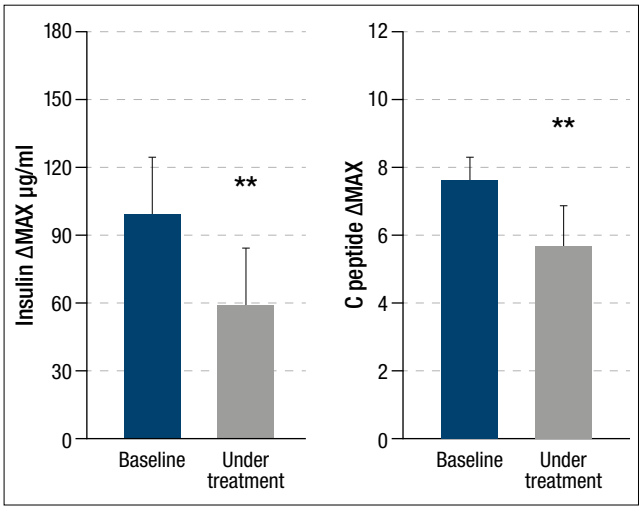


Table 1 Hormonal characteristics of all PCOS patients under study.

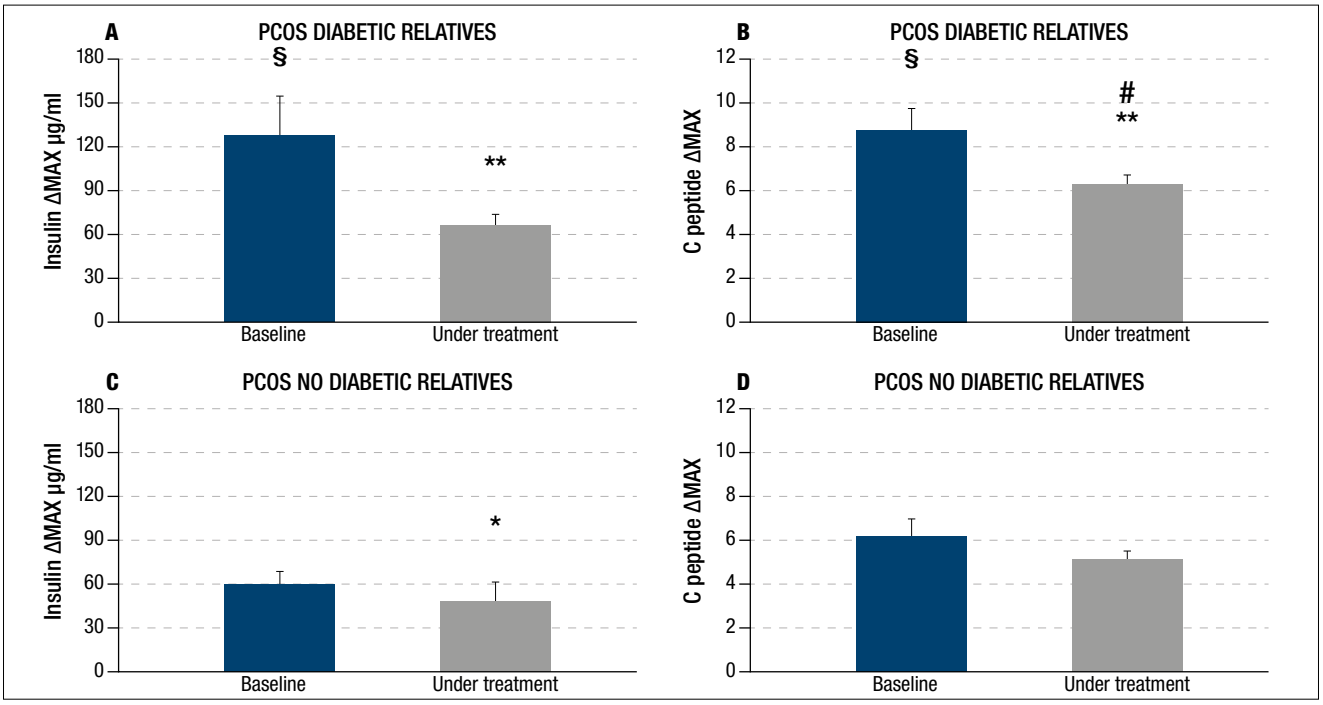
PCOS patients n=30	LH mIU/ml	FSH mIU/ml	Estradiol pg/ml	A ng/ml	Total T ng/ml	Insulin µU/ml	Glucose mg/dl	Tryglicerides mg/dl	Total Cholesterol mg/dl	HDL mg/dl	LDL mg/dl	GOT U/l	GPT U/l	BMI	HOMA index
Baseline	13,5±1,5	5,8±0,5	47,1±5,9	26,8±0,15	0,6±0,04	14,1±2,6	88,2±2,7	126,2±22	182,7±9,9	50,3±4,5	112±12,7	24,5±2,6	29,7±4,7	31,5±1,4	3,12±0,6
Under treatment	8,6±0,9	5,6±0,5	62,8±14	2,2±0,15	0,4±0,04	9,5±1,3	84,5±2,2	99±13,3	174,3±7,3	55,5±3,3	104±8,4	19,8±1,8	24,7±2,5	30,4±1,3	2,1±0,3
p level vs baseline	0,01			0,0003		0,003					0,04			0,0006	0,002

Table 2 Hormonal characteristics of PCOS patients according to the presence or absence of diabetic relative(s).

Diabetic relatives n=18	LH mIU/ml	FSH mIU/ml	Estradiol pg/ml	A ng/ml	Total T ng/ml	Insulin µU/ml	Glucose mg/dl	Tryglicerides mg/dl	Total Cholesterol mg/dl	HDL mg/dl	LDL mg/dl	GOT U/l	GPT U/l	BMI	HOMA index
Baseline	12.8±1,6	5.4±0.5	56±10.5	280.8±11	0.6±0.03	14±2.8	87,5±3	118,7±18	188,5±10,3	52,8±4,8	116,7±12,2	27.4±2,4	33.5±3.9	32.5±1.5	3.1±0.7
p vs NO diabetic												0.03	0.02		0.05
Under treatment	8.7±1.1	5.2±0.5	62.6±14	240±23	0.4±0.03	11±2.2	87,8±3.2	97,1±13.1	175±10,1	59,1±5	103±12	20.8±1.2	21.5±3	31.1±1.5	2.5±0.6
p level vs baseline	0.02			0.02		0.02		0.009	0.05		0.02	0.006	0.004	0.0001	0.03

NO Diabetic relatives n=12	LH mIU/ml	FSH mIU/ml	Estradiol pg/ml	A ng/ml	Total T ng/ml	Insulin µU/ml	Glucose mg/dl	Tryglicerides mg/dl	Total Cholesterol mg/dl	HDL mg/dl	LDL mg/dl	GOT U/l	GPT U/l	BMI	HOMA index
Baseline	13.4±2.2	5.9±0.6	48.1±7.1	233.3±18	0.6±0.06	9.5±1.3	83.7±2.5	108.7±24	177.7±12	52,7±2.4	107.2±11.1	19.8±2.4	19.5±3.6	30±2.9	2.2±0.3
Under treatment	8.5±1.6	6.2±0.9	41.7±3.6	193.1±20	0.4±0.08	7.4±1.2	80.1±2.2	101.5±27	173.3±12	51±4	106.1±12.7	17.8±1.2	18.2±2.7	30.7±2.8	1.6±0.3
p level vs baseline	0.05			0.01		0.01									0.01

Figure 2 Maximal insulin and c-peptide response (Δmax) to OGTT in PCOS patients subdivided according to the presence or absence of familial diabetes. Insulin and c-peptide Δmax were found to be higher in PCOS patients with familial diabetes.
Panel A: ** p<0.01 vs baseline; § p<0.03 vs no diabetes (panel C); Panel B: ** p<0.03 vs baseline; § p<0.05 and # p<0.04 vs no diabetes (panel D).
Panel C: * p<0.05 vs baseline.



Discussion

The present study reported improvements in hormonal and metabolic parameters in obese PCOS patients administered DCI+ALA. Moreover, our data support the relevance of the presence of familial diabetes, since this predisposes to greater metabolic impairment and liver dysfunction.

Insulin resistance (IR) is a frequent finding in PCOS patients but it is not completely related to being overweight or obese, since it also occurs in normal weight PCOS subjects [12, 19, 20]. In fact, higher occurrence of IR is classically a feature of those patients who have familial diabetes [12, 20]. Metformin has been demonstrated to reduce IR but due to its side effects, especially in subjects needing higher dosages [12], alternative strategies have been developed, such as the use of MYO, DCI and ALA. These compounds have been demonstrated to improve IR by increasing the efficiency of post-receptor signalling of insulin [11, 12, 13, 16, 20]. Previous studies demonstrated that MYO administration was effective in improving insulin sensitivity in hyperinsulinemic PCOS patients [11], similarly to what was observed when using DCI [13] or ALA [16], having no side effects and a marked clinical impact on metabolic dysfunction and hormonal impairment.

The present data are clearly in line with such studies, and add, to their findings, results observed when DCI and ALA are used in combination. According to a recent review MYO or DCI can be clinically helpful whether absence or presence of familial diabetes has been disclosed [14]. Moreover, previous studies reported that DCI administration [13] was able to reduce IR in PCOS subjects, including those with familial diabetes [13], similarly to the use of ALA [16]. Our data are in perfect agreement with previous observations demonstrating that the combination of DCI and ALA greatly improved insulin sensitivity in all subjects, independently of the presence or absence of familial diabetes [13, 16]. However, subdivision of the patients on the basis of this latter criterion allowed us to disclose greater efficiency of the treatment in PCOS patients with familial diabetes. In the other group, the treatment improved hormonal parameters, but less evidently than in PCOS patients with familial diabetes. In fact, PCOS patients with familial diabetes showed not only improved plasma LH, insulin and A concentrations, but also improved levels of triglycerides, total cholesterol, LDL, GOT and GPT.

Our evaluation of ΔMax for insulin and c-peptide under OGTT revealed that though both insulin and c-peptide decreased after the treatment, PCOS patients with familial diabetes had a higher maximal responses (ΔMax) of insulin and c-peptide to glucose load in baseline conditions, and that the DCI+ALA administration decreased ΔMax at a higher rate in this group of PCOS patients. In fact, PCOS patients with no familial diabetes showed no significant changes in c-peptide ΔMax after the combination treatment, thus suggesting that familial predisposition to diabetes underlies, to a certain extent, the metabolic impairment, worsening the IR and the compensatory hyperinsulinemia, probably by increasing the pancreatic secretion/function of the Langerhans islets, even though impaired insulin metabolic clearance cannot be excluded.

The hormonal and metabolic improvements we observed confirm a previous report by Cianci A et al. [21], even though those authors used higher doses of DCI and ALA. As an additional feature, our report described the efficacy of the treatment on liver function, since we observed a significant reduction of hepatic enzyme levels (i.e. GOT and GPT) in the PCOS patients with familial diabetes. This observation is superimposable on

what was previously described when administering ALA alone [16]. In fact, the presence of type II diabetes downregulates the expression of lipoic acid synthase (LASY), responsible for ALA synthesis in mammalian mitochondria [22, 23], thus reducing endogenous ALA synthesis and leading to the lower glucose uptake in skeletal muscle cells that is at the basis of IR [23]. Endogenous ALA modulates glucose utilization through the increase of adenosine monophosphate-activated protein kinase in skeletal muscles [12], and thus by increasing glucose-transporter-4 levels [24, 25]. These data support the fact that having familial diabetes predisposes to impaired endogenous synthesis of both ALA and DCI, related to defective expression/function of LASY and epimerase [12, 16] respectively.

The combination DCI and ALA modulated, at the same time, both hormonal and metabolic aspects. ALA has recently been reported to act on specific metabolic indexes and to exert a good hepatic protective action with no improvement of reproductive hormonal profiles in PCOS subjects, independently of familial diabetes status [17]. Our data report, for the first time, that the DCI+ALA combination has a full effect in PCOS patients, since it shows a hepatic protective action in addition to metabolic and hormonal effects. Indeed, this combination might be effective in preventing not only the risks related to IR and compensatory hyperinsulinemia, but also the risk of developing non-alcoholic fatty liver disease (NAFLD) [26]. A recent review stated that NAFLD is very frequent in PCOS patients [26] and the combination of PCOS with obesity and IR is a dangerous cocktail that, over time, triggers not only NALFD but also the occurrence of type II diabetes [26, 27].

In conclusion, the combined DCI+ALA regimen, at the low dosages we used, was effective in improving both hormonal (related to DCI) and metabolic (related to both DCI and ALA) parameters. The present study clearly supports the need for an accurate anamnestic investigation, so as to better choose the most effective combination treatment strategy.

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