

Effects of a Combination of Alpha Lipoic Acid and Myo-Inositol on Insulin Dynamics in Overweight/Obese Patients with PCOS

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Received date: Jun 18, 2014, Accepted date: Sep 26, 2014, Published date: Sep 30, 2014

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Abstract

Myo-inositol increases insulin sensitivity in insulin resistant patients with PCOS since it improves the insulin post-receptor pathways. Since previous reports suggested that also alpha lipoic acid has specific positive effects on glucose control, we aimed to evaluate the specific effects of a combination of alpha lipoic acid and myo-inositol on insulin resistance in obese patients with PCOS.

We studied a group of obese PCOS patients (n=34, BMI= 30.1 ± 0.9) according to the revised 2003 Rotterdam consensus diagnostic criteria. Among the PCOS patients, 16 out of 34 had diabetic type II relatives (parents and/or grandparents). Patients were administered a combination of alpha lipoic acid (400 mg) and myo-inositol (1 gr.) (Sinopol, Laborest, Italy) every day for at least 12 weeks. Patients underwent to baseline hormone determination and to an oral glucose tolerance test (OGTT) before and at the 12th week of treatment.

After the treatment interval, HOMA index decreased significantly as well as the glucose-induced insulin response with no changes of BMI. Interestingly the treatment did not change insulin dynamics in normo-insulinemic PCOS while significant insulin decrease was observed in hyperinsulinemic PCOS patients. 87.5% (14 out of 16) of the PCOS patients with diabetic relatives resulted to be among the hyperinsulinemic patients. Hyperinsulinemic PCOS patients showed the significant decrease of the insulin plasma levels (from 14 ± 2.1 to 9.5 ± 0.8 µU/ml, p<0.05), of HOMA index (from 3.3 ± 0.4 to 2.1 ± 0.1, p<0.05) and showed the significant decrease of insulin response to glucose load.

In conclusion, the combination of alpha lipoic acid plus MYO was effective in improving insulin sensitivity in obese PCOS patients that resulted to be hyperinsulinemic under OGTT. Moreover the more peculiar and relevant positive changes were observed in obese PCOS with diabetic first grade relatives.

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common diseases affecting women during their reproductive life since it affects 5-21% of them [1]. The Consensus Meeting in Rotterdam [2,3] defined that PCOS requires two or more of the following requirements: chronic anovulation disorder (oligo- to anovulation or amenorrhea), presence of hyperandrogenism (clinical and/or in laboratory tests) and ultrasound evidence of polycystic ovaries. Recently, an additional feature seems to be relevant for the syndrome: insulin resistance. Indeed, several studies reported that insulin resistance is common in PCOS patients, regardless of the body mass index (BMI). In fact, compensatory hyperinsulinemia due to insulin resistance occurs in approximately 80% of women with PCOS and central obesity as well as in 15–30% of lean women diagnosed with PCOS [3,4].

The cause of the insulin resistance observed in PCOS women is not yet clear, especially in lean patients, but a post-receptor defect that could affect glucose transport has been proposed [5-7]. Obesity exacerbates insulin resistance and it is a fundamental causal factor in the pathogenesis of anovulation and hyperandrogenism.

Beyond the reproductive consequences of PCOS, these patients are at high risk to develop metabolic abnormalities, mainly type II diabetes, starting from the hyperinsulinemia they might develop. Indeed, conversion from normal glucose tolerance to hyperinsulinemia due to impaired glucose tolerance and later to type II diabetes is accelerated in PCOS patients [8-13]. For this reason, many organizations recommend screening women with PCOS for glucose intolerance [14-16] at least with a 2-hour oral glucose tolerance test (OGTT). Based on such indications, early detection and lifestyle changes or pharmacological interventions have been reported to delay or block the development of type II diabetes [5,17].

Among the many possible intervention in PCOS, other than pharmacological treatments such as the use of metformin (5), various integrative compounds have been proposed such as the two isoforms of inositol, that is myo-inositol (MYO) and d-chiro-inositol (DCI), and alpha lipoic acid (ALA). Both inositols and ALA were reported to be effective in reducing the insulin resistance in PCOS patients [17-19]. While inositols are involved in the structure of the post receptor transduction of the signal induced by the linkage of insulin on its receptor [20], ALA has been demonstrated in the animal model to increase glucose utilization through the increase of adenosine monophosphate-activated protein kinase (AMPK) in skeletal muscles

[21] thus increasing glucose-transporter-4 (GLUT-4) [22,23]. It is interesting to observe that also metformin treatment seems to activate AMPK [24]. On such basis and evidences, we aimed to evaluate the effects of a combination of inositol (MYO) and ALA on insulin sensitivity and hormonal parameters in a group of overweight/obese PCOS patients.

Materials and Methods

Subjects

Among the many PCOS patients attending our outpatients Clinic between June 2012 and December 2013, a group of 44 PCOS overweight/obese patients, requiring treatment for their condition, were recruited for this study after informed consent. These patients were selected among those attending the Gynecological Endocrinology Centre at the University of Modena and Reggio Emilia, Italy, according to the revised 2003 Rotterdam consensus diagnostic criteria for PCOS.

The diagnosis of PCOS was based on the association of at least two of the following criteria: a) oligomenorrhea with inter-menstrual intervals longer than 35 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 e) absence of enzymatic adrenal deficiency and/or other endocrine disease, including diabetes, f) normal PRL levels (range 5-25 ng/ml), g) no hormonal treatment for at least 6 months before the study, h) body mass index (BMI) above 25.

Among the 44 patients, 8 preferred not to participate and/or requested a contraceptive method and were excluded from the study. Among the remaining 36 PCOS patients, 2 became pregnant within 6-8 weeks after starting the study. The patients finally considered for the study were 34 (26.4 ± 0.8 yrs). All the patients were interviewed in regards to the presence of one or more diabetic relatives (parents and/or grandparents). From this anamnestic investigation 16 patients out of 34 reported diabetic relatives. All patients were treated with a combination of alpha-lipoic acid (400 mg) and myo-inositol (MYO) 1 gr. every day (Laborest, Nerviano, Milan, Italy), every morning around 10 a.m., for at least 3 months (12 weeks). No changes of life style or diet were required from the patients. All patients were studied the first time on day 3-6 of the menstrual cycle. The post treatment endocrine control was performed at least at the 12th week of treatment or few days later, so that to be on day 3-6 of the first menstrual cycle occurring after the treatment interval.

All patients were evaluated for LH, FSH, estradiol (E2), progesterone (P), androstenedione (A), insulin; HOMA index was

computed to estimate the sensitivity to insulin [25]. Oral glucose tolerance test (OGTT), for insulin and glucose determinations, was performed sampling before and 90 minutes after the oral assumption of 75 gr. of glucose, before and after 12 weeks of treatment. Hyperinsulinemic response is recognised when insulin plasma levels are above 50 µU/ml 90 minutes from glucose load [26]. The mean treatment interval was 90.5 ± 4 days (mean ± standard error of the mean [SEM]), being the range of 89-110 days. The study protocol was approved as observational study by the Human Investigation Committee of the University of Modena and Reggio Emilia, Italy (registration n. 181/12).

Assay

All samples were assayed in duplicate in the same assay. Plasma LH and FSH concentrations were determined using a previously described immunofluorimetric assay (IFMA) [27]. Intra-assay and inter-assay coefficients of variation were 5.1 and 7.3%, respectively. Plasma E2, P, A were determined by radioimmunoassay (Radim, Pomezia, Rome, Italy) as previously described [28]. Within- and between assay coefficients of variation were 4.1% and 9.5%. Plasma insulin was determined using an immunoradiometric assay (Biosource Europa S.A., Nivelles, Belgium). Within- and between-assay coefficients of variation were 4.5% and 11.7%.

Statistical analysis

As in previous studies [29], overweight and obese PCOS patients were considered all together. Data are expressed as mean (SEM). We tested data for significant differences between groups, after analysis of variance (one-way ANOVA), using Student's t-test for paired data (baseline vs. under treatment). HOMA index was computed to estimate the sensitivity to insulin [25] 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 insulin resistance (HOMA-IR) as (fasting insulin mU/l) × (fasting glucose mmol/l)/22.5 [25]. The cutoff value we used is 2.71 as previously stated [25,26] while it is 2.5 for children and adolescents [30].

Results

All hormonal parameters of patients under study are summarized in Table 1. Insulin plasma levels and HOMA index resulted significantly reduced after the treatment interval. BMI decreased but did not reach significance. In addition, LH plasma levels, LH/FSH ratio (Table 1) and insulin response to the oral glucose tolerance test (OGTT) (Figure 1) were significantly decreased.

PCOS (n=34)	LH mIU/ml	FSH IU/ml	LH/FSH	E2 pg/ml	P ng/ml	A ng/100 ml	Insulin µU/ml	HOMA index	BMI
Baseline	11.2 (0.6)	6.4 (0.1)	1.8 (0.1)	45.3 (2.5)	0.4 (0.02)	194.4 (18.6)	13.4 (1.8)	2.9 (0.4)	30.1 (0.9)
Under treatment	8.3 (0.5)***	6.6 (0.3)	1.3 (0.07)**	39.4 (3.2)	0.4 (0.04)	205 (10.5)	9.5 (0.7)*	2.0 (0.1)*	29.6 (0.6)

Table 1: Biochemical parameters of PCOS patients (n=34) before and under treatment. Data are shown as mean (SEM).

LH: luteinizing hormone; FSH: follicle stimulating hormone; E2: estradiol; P: progesterone; 17OHP: 17 hydroxyprogesterone; A: androstenedione, BMI: body mass index.

***p<0.001; **p<0.02; *p<0.05 vs baseline

When we subdivided the PCOS patients in hyperinsulinemic (n=28) and normoinsulinemic (n=6) patients according to the insulin response to the OGTT (Table 2) both groups demonstrated the reduction of LH and LH/FSH ratio but only hyperinsulinemic PCOS showed the significant decrease of HOMA index. Normoinsulinemic patients showed a perfectly normal HOMA index both before and after the treatment interval. Interestingly, when considering the insulin response to glucose load only the hyperinsulinemic PCOS patients showed a reduced insulin release (Figure 2) while no change was observed for the normoinsulinemic subjects.

Considering that some patients reported diabetic first-grade relatives, we reconsidered the hormonal profiles (Table 3) and the insulin response (Figure 3) subdividing our PCOS patients into two groups: those with diabetic relatives (n=16) and those with no diabetic relatives (n=18). Patients with diabetic relatives showed significant reduction of LH, LH/FSH ratio, insulin and HOMA index while patients who had no diabetic relatives had only the reduction of LH and LH/FSH ratio (Table 3). It is to note that 14 out of 16 (87.5%) of the PCOS patients with diabetic relatives were hyperinsulinemic under OGTT and they represented 57.1% of all the hyperinsulinemic PCOS we studied. Both groups showed the significant reduction of the insulin response under glucose load (Figure 3) but only the PCOS with diabetic relatives showed a significantly reduced insulin levels at time 0, similarly to hyperinsulinemic PCOS (Figure 2).

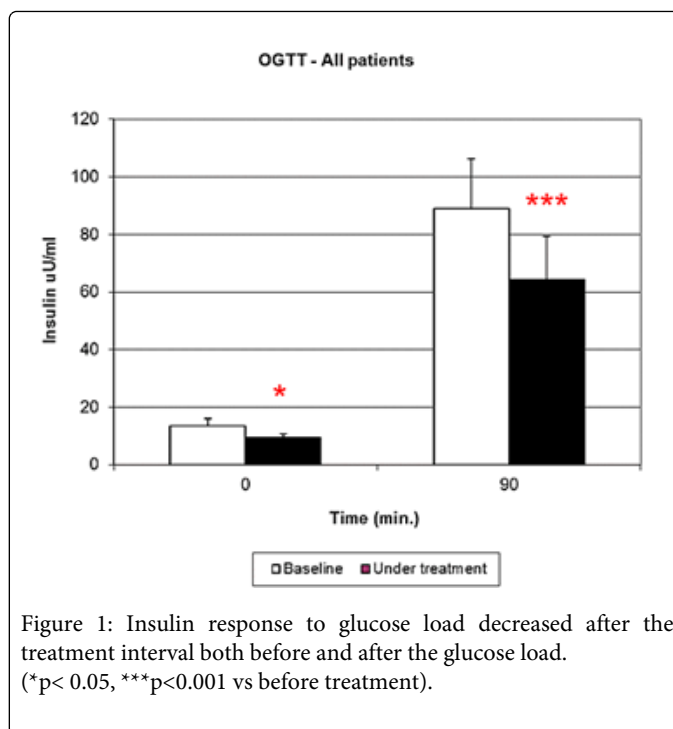


Figure 1: Insulin response to glucose load decreased after the treatment interval both before and after the glucose load. (*p<0.05, ***p<0.001 vs before treatment).

PCOS Hyperinsulinism (n=28)	LH mIU/ml	mIU/ml	LH/FSH	E2 pg/ml	P ng/ml	A ng/100 ml	Insulin µU/ml	HOMA index	BMI
Baseline	12.4 (0.6)	6.1 (0.1)	2.0 (0.1)	53.5 (5.1)	0.5 (0.03)	254.0 (21)	14.0 (2.1)	3.3 (0.4)	29.8 (1.5)
Under treatment	9.0 (0.6)**	6.6 (0.4)	1.4 (0.06)***	42.4 (4.1)	0.5 (0.05)	247.0 (11)	9.5 (0.8)*	2.1 (0.1)**	29.0 (2.5)
PCOS No hyperinsulinism (n=6)	LH mIU/ml	FSH mIU/ml	LH/FSH	E2 pg/ml	P ng/ml	A ng/100 ml	Insulin µU/ml	HOMA index	BMI
Baseline	8.6 (1.1)	7.2 (0.6)	1.2 (0.1)	46.0 (7.7)	0.3 (0.06)	143.3 (20.4)	6.0 (0.5)	1.3 (0.2)	30.3 (1.4)
Under treatment	6.3 (1.2)*	6.4 (1.2)	0.9 (0.1)*	33.3 (1.3)	0.3 (0.06)	160.0 (25)	6.8 (1.5)	1.5 (0.3)	29.4 (1.7)

Table 2: Biochemical parameters of PCOS patients subdivided according to the insulin response to OGTT, data are shown as mean (SEM). LH: luteinizing hormone; FSH: folliclestimulating hormone; E2: estradiol; P: progesterone; 17OHP: 17 hydroxyprogesterone; A: androstenedione; BMI: body mass index. ***p<0.001; **p<0.01; *p<0.05 vs baseline

PCOS Familiar diabetes (n=16)	LH mIU/ml	FSH mIU/ml	LH/FSH	E2 pg/ml	P ng/ml	A ng/100 ml	Insulin µU/ml	HOMA index	BMI
Baseline	12.0 (0.9)	6.0 (0.2)	2.0 (0.1)	50.2 (8.1)	0.5 (0.04)	233.0 (26)	11.6 (1.3)	3.2 (0.5)	31.4 (2.3)
Under treatment	8.9 (0.7)*	6.2 (0.2)	1.4 (0.07)	48.0 (5.6)	0.5 (0.06)	220.0 (19)	7.5 (0.6)*	1.8 (0.1)*	30.0 (2.2)
PCOS No familiar diabetes (n=18)	LH mIU/ml	FSH mIU/ml	LH/FSH	E2 pg/ml	P ng/ml	A ng/100 ml	Insulin µU/ml	HOMA index	BMI
Baseline	11.7 (0.8)	6.5 (0.2)	1.8 (0.1)	50.7 (3.7)	0.4 (0.04)	242.5 (20)	10.3 (2.4)	2.8 (0.6)	28.3 (1.0)

Under treatment	7.6 (0.8) **	6.8 (1.1)	1.2 (0.1)**	30.8 (2.3)*	0.35 (0.06)	223.0 (12)	10.3 (1.2)	2.2 (0.2)	28.2 (0.9)
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Table 3: Biochemical parameters of PCOS patients subdivided according to the presence (upper panel) or absence (lower panel) of diabetic relatives (mean ± SEM).

LH: luteinizing hormone; FSH: follicle stimulating hormone; E2: estradiol; P: progesterone; 17OHP: 17 hydroxyprogesterone; A: androstenedione; BMI: body mass index. **p<0.01; *p<0.05 vs baseline

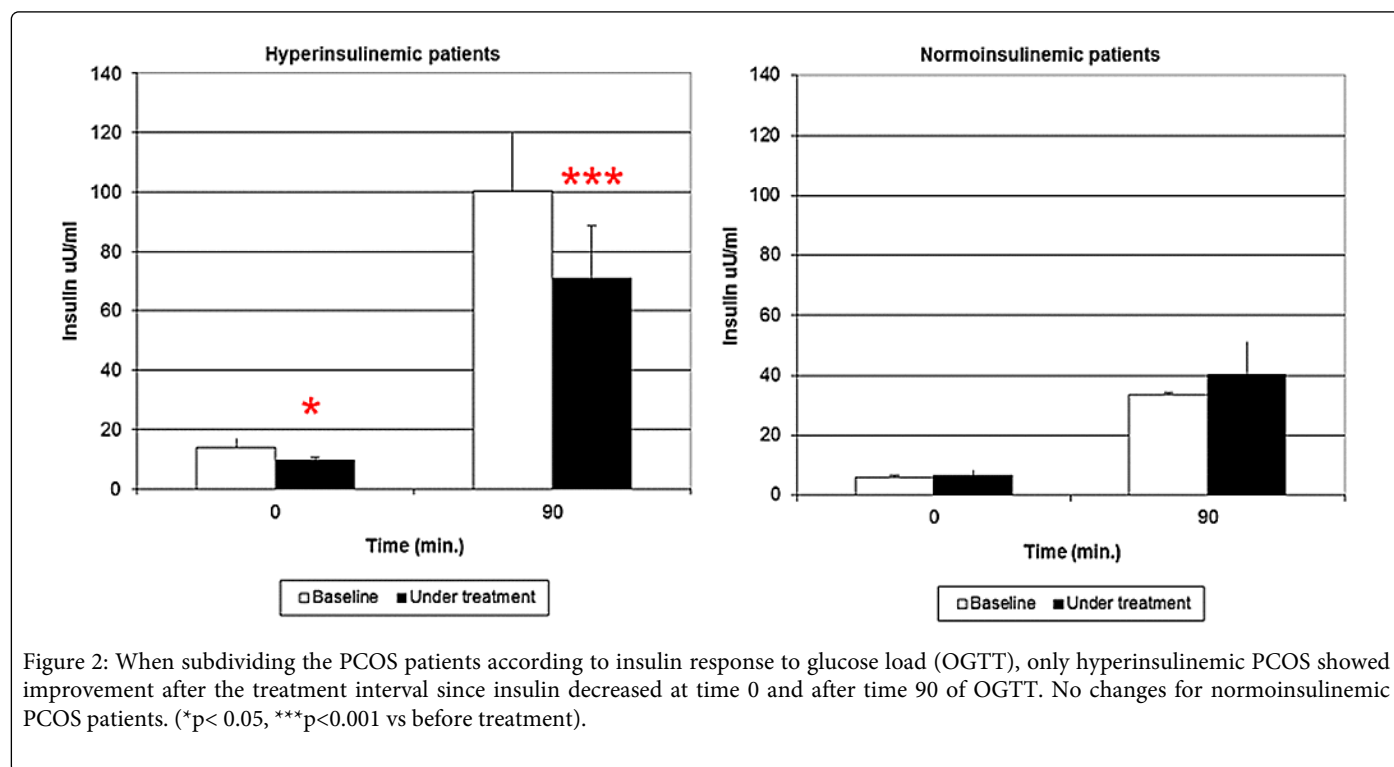


Figure 2: When subdividing the PCOS patients according to insulin response to glucose load (OGTT), only hyperinsulinemic PCOS showed improvement after the treatment interval since insulin decreased at time 0 and after time 90 of OGTT. No changes for normoinsulinemic PCOS patients. (*p< 0.05, ***p<0.001 vs before treatment).

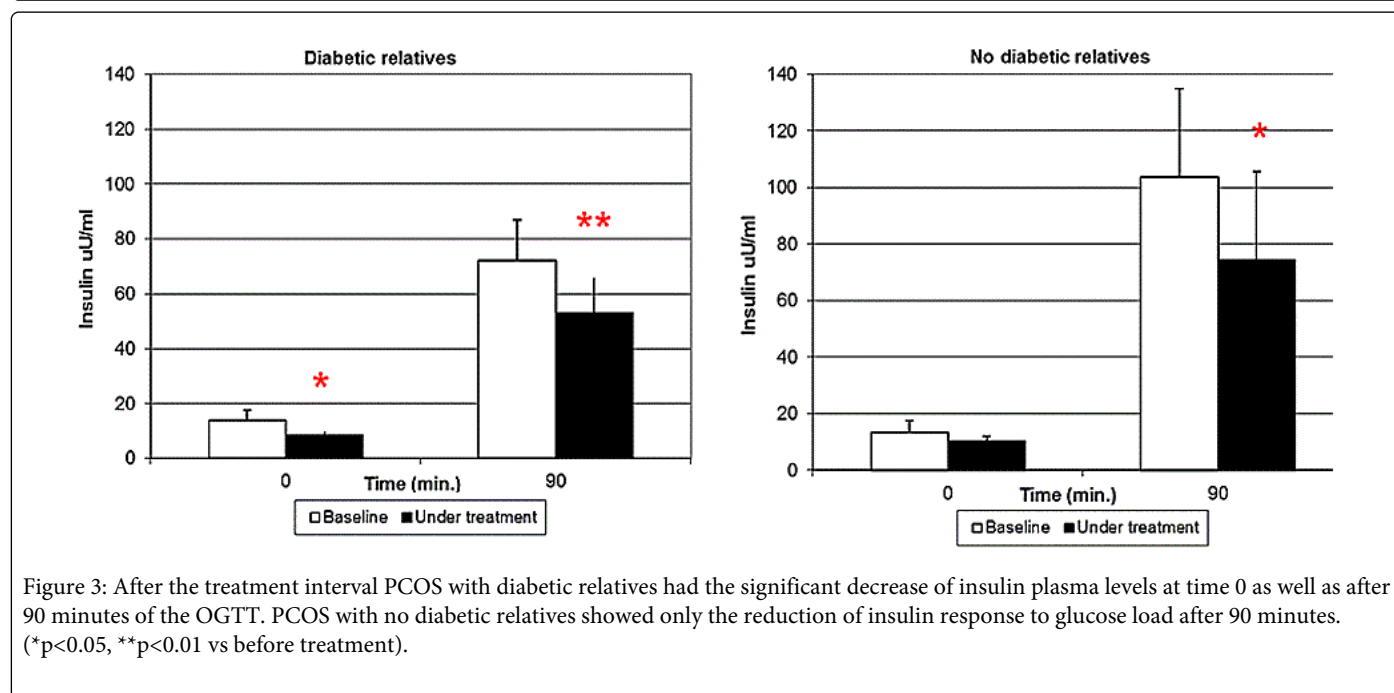


Figure 3: After the treatment interval PCOS with diabetic relatives had the significant decrease of insulin plasma levels at time 0 as well as after 90 minutes of the OGTT. PCOS with no diabetic relatives showed only the reduction of insulin response to glucose load after 90 minutes. (*p<0.05, **p<0.01 vs before treatment).

Discussion

This manuscript reports the ability of a combination of alpha lipoic acid (ALA) and myo-inositol (MYO) to modulate and reduce insulin resistance and glucose-load induced insulin hyper secretion in a group of PCOS patients, improving also gonadotropin secretion.

Recently we reported the efficacy of MYO [26,31,32] as well as of DCI [18] to modulate insulin sensitivity and hormonal profiles in PCOS patients and we demonstrated that when there is the presence of first grade diabetic relatives the putative DCI administration seems to be more effective than MYO in the control of insulin sensitivity [18]. Such hypothesis was enforced by the fact that growing evidences suggest that a deficiency of d-chiro-inositol (DCI) containing IPG might be at the basis of insulin resistance, so frequent in PCOS patients. Moreover, recently it has been reported that PCOS patients have abnormally high urinary clearance of DCI [6] and that metformin administration in obese PCOS patients improves the release of DCI-IPG mediator [33]. Such observations have clearly suggested that an impairment of IPG mediator(s) might be one of the putative casual factors of the insulin resistance and of the compensatory hyperinsulinemia that most PCOS patients show. It is relevant to remember that DCI is synthesized by an epimerase that converts MYO into DCI and that, depending on the specific needs of the two molecules, each tissue has a typical conversion rate [34,35]. Such MYO-to-DCI conversion seems to be insulin dependent since ratios of MYO to DCI is increased to about 10- to 20-fold in type I diabetic patients, in first-degree relatives of type II diabetic patients and in type II diabetic subjects [34].

On such basis, it seemed logic to test the efficacy of a combination of alpha lipoic acid (ALA) and MYO to evaluate what improvement might be induced by this combination.

Our data demonstrated that only PCOS patients with an abnormal HOMA index showed a significant improvement undergoing to the treatment of ALA+MYO. In fact, though being a very limited amount of patients, normoinsulinemic PCOS, though obese, did not show any modification of HOMA index and of insulin response to glucose load, similarly to what recently reported [26]. Moreover, it is interesting to observe that among the hyperinsulinemic PCOS there was the highest rate (87.5%) of patients with diabetic first grade relatives. As additional fact, under ALA+MYO administration, only PCOS patients with diabetic relatives showed the significant reduction of insulin plasma levels in fasting conditions. This is interesting since it suggests that in these patients with diabetic relatives ALA administration plays a specific and positive role, which is different from in the other PCOS patients with no diabetic relatives. Such data fit perfectly with what previously demonstrated for ALA [19,36,37]. In fact, ALA administration has been reported to be effective on reducing insulin resistance in PCOS patients [19], with also specific effects on triglycerides plasma levels. In animal models and in humans the presence of diabetes type II downregulates the expression of lipoic acid synthase (LASY) which is responsible of the synthesis of ALA inside the mitochondria of mammals [36,37]. Reduced ALA synthesis results in a decrease in mitochondrial lipoic acid that induces a lower glucose uptake in skeletal muscle cells that is at the basis of insulin resistance [37]. In fact, ALA modulates glucose utilization through the increase of adenosine monophosphate-activated protein kinase (AMPK) in skeletal muscles [21] thus increasing glucose-transporter-4 (GLUT-4) [23,24] in the animal model. It is interesting to observe that also metformin treatment seems to activate AMPK [24].

Unfortunately, the study protocol was approved with no ALA- or MYO-only treated group. This would have given a more detailed insight on the efficacy of these integrators. Nevertheless, considering other reports on MYO or ALA administration, our data support the hypothesis that not only MYO is effective in overweight PCOS, as previously reported [26,31] but the addition of ALA improved glycemic control. In fact as previously reported [37] a consistent part of the insulin sensitivity is related to the LASY expression and activity. In animal models as well as in diabetes patients the abnormal LASY function decreases ALA synthesis affecting insulin sensitivity [37].

Our data let us infer that these mechanism(s) are at the basis of the efficacy of the ALA+MYO combination on all overweight/obese PCOS patients. In addition, the fact that those patients who had diabetic relative(s) showed more relevant and significant changes of plasma insulin levels and HOMA index sustain the hypothesis that the LASY abnormal expression might be a relevant casual co-factor of the insulin resistance and of the compensatory hyperinsulinemia. Indeed a higher improvement on insulin sensitivity is given by ALA administration, as demonstrated previously [19,37] and by the greater availability of MYO, due to the treatment, modulated insulin response, similarly to what previously reported in normal weight [32] and in obese PCOS patients [26,31]. According to our data the concomitant presence of these two compounds modulate insulin-induced pathways. In these PCOS patients, the combination of ALA+MYO improved the insulin response to glucose load and those who had diabetic relatives such effect showed up early, that is in fasting conditions. These observations let us infer that the presence of ALA together with MYO is highly effective mainly in obese hyperinsulinemic PCOS who had diabetic relatives since probably restores the reduced ALA endogenous concentrations, improving ALA modulatory effects on insulin sensitivity.

Finally yet importantly, it is relevant to point out the significant changes of the mean LH plasma levels, independently from the subdivision adopted on the patients. Such data give clear relevance to the fact that insulin and/or the intracellular IPG-mediator modulate pituitary function in terms of LH secretion and sustain the hypothesis that metabolic pathways and its hormonal modulators play a primary role on endocrine glands activity. These data are in agreement with previous studies [26,31,32] and disclose the fact that LH changes occur also in normoinsulinemic PCOS patients and/or without first grade diabetic relatives. Though we did not assay testosterone plasma levels, the androgenic milieu did not change since androstenedione plasma levels did not change. This observation is not in agreement with previous data on MYO administration in overweight PCOS patients [26,31] but the population of patients we studied was different and had not a so elevated androstenedione plasma levels as the above mentioned studies. This observation confirms that PCOS may show some variability of one or more of the many hormones that have been demonstrated to be part of the syndrome.

In conclusion, though the number of the patients studied represents a limiting factor, our data support the hypothesis that integrative administration of ALA+MYO combination improved insulin sensitivity in overweight/obese PCOS. In addition, our data suggest that ALA might be a relevant helper for the insulin resistance and metabolic impairment that characterize a high percentage of PCOS patients, especially if they have diabetic relatives. Though lifestyle modification should be the first significant change to adopt [17] to avoid the evolution of PCOS towards the more dangerous and risky metabolic syndrome [38], the use of appropriate feeding integration(s)

might help a lot. More studies should be addressed to better disclose the role of ALA on the biochemical control of the metabolic pathways in PCOS patients.

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