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A multicenter clinical study with myo-inositol and alpha-lactalbumin in Mexican and Italian PCOS patients

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Abstract. – OBJECTIVE: This open-label non-randomized clinical study aimed at evaluating the effects of myo-inositol plus alpha-lactalbumin in two groups of PCOS women, treated in Mexico and Italy. Alpha-lactalbumin was used being effective in increasing myo-inositol intestinal absorption. This effect is very useful in greatly reducing the therapeutic failure of myo-inositol in some patients (inositol resistant subjects).

PATIENTS AND METHODS: The study involved 34 normal weight or overweight patients (14 in Mexico and 20 in Italy), aged 18 to 40 years, with anovulation and infertility > 1 year and insulin resistance diagnosed by HOMA-Index. Patients were administered orally with 2 g myo-inositol, 50 mg alpha-lactalbumin, and 200 μg of folic acid twice a day for 6 months. Controls were the same patients at t0 (baseline). The primary outcome was HOMA-index decrease after 3 and 6 months of treatment. Other parameters monitored were BMI, progesterone, LH, FSH, total testosterone, free testosterone, androstenedione, total cholesterol, HDL, LDL, triglycerides.

RESULTS: Recovery was general, and its relevance was higher when the starting point was further away from the normal range. The most important results were obtained with insulin, HOMA-index, LH, and androstenedione. No significant adverse effects were detected in both groups of patients.

CONCLUSIONS: This clinical trial demonstrated for the first time that myo-inositol and alpha-lactalbumin improve important parameters in PCOS patients characterized by different metabolic profiles.

Key Words:
Alpha-lactalbumin, Insulin-resistance, Microbiota, Myo-inositol, Polycystic Ovary Syndrome.

Authorization number 0020/19/R. Released by the Ethics Committee of the Juárez Hospital, Ciudad de México. Board Affiliation 14 CI 09005115 (affiliation number in COFEPRIS). Study registration number NCT04645745 of the ClinicalTrials.gov registry.

Introduction

Based on the Rotterdam criteria (2003), Polycystic Ovary Syndrome (PCOS) is diagnosed when the patients show two of the following clinical and endocrine characteristics: chronic ovulatory disorder, clinical and/or biochemical hyperandrogenism, and polycystic ovaries[1]. Although these criteria do not include insulin resistance (IR), it is well-known that deregulation of insulin sensitivity, and/or abnormalities in glucose metabolism, are common features in several PCOS women[2]. The primary intervention involves a change in diet and lifestyle that can act efficaciously in some patients, restoring ovary function and warding off possible PCOS-related consequences. Nevertheless, in many cases, physicians have to prescribe specific therapies based on drugs or dietary supplements. The main feature of these compounds is their insulin-sensitizing, or insulin-mimetic

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action, necessary to significantly reduce hyperinsulinemia. Metformin and thiazolidinediones are common drugs used for PCOS[3]. However, they induce several and sometimes serious side-effects[4,5]. Instead, among the dietary supplements myo-inositol (MI) and, to a lesser extent, its stereoisomer D-chiro-inositol (DCI) deserves to be considered. MI exerts an insulin sensitizing action[6], being, in the form of MI–IPG, one of the second messengers of this hormone[7]. Moreover, MI (as Inositol trisphosphate) in the ovary acts as one of the second messengers of follicle-stimulating hormone (FSH)[8], without any direct involvement of DCI. MI levels in the mammalian female reproductive tract are considerably higher than in blood, and this suggests the existence of a specific role played in the ovary by MI, essential for correct oocyte maturation[9]. Therefore, the therapeutic use of MI in patients affected by IR is justified by its physiological role. Of note, inositols are side-effect free at therapeutic doses, as also testified by Food and Drug Administration (FDA)[10,11], providing an essential advantage over the previously mentioned drugs. Some studies demonstrated that MI plays beneficial roles on oocytes, whereas an increase of DCI is detrimental[12,13]. This is very important in consideration that PCOS women often show a poor oocyte quality. However, MI treatment was not always fully effective in all patients affected by this syndrome with its frequently associated conditions such as anovulation, subfertility, or infertility. These subjects were defined inositol resistant. Various clinical studies found that between 28% and 38% of PCOS patients do not respond satisfactorily to MI treatment[10,14]. It is believed that inositol resistance largely derives from its reduced or absent intestinal absorption due to conditions that are not yet clear or foreseeable, such as, for example, dysbiosis, obesity, and chronic intestinal diseases. Alpha-lactalbumin (alpha-LA), a milk protein with low immunogenicity, was demonstrated to be useful to overcome this drawback. Among its manifold physiological and therapeutic roles, we call attention to its ability to increase the passage of steroids or metals (i.e., vitamin D, iron) through biological membranes[15,16]. Furthermore, this molecule is very safe, so much so that FDA included it in the list of GRAS compounds[17]. Two studies demonstrated that alpha-LA can increase the MI intestinal absorption and, consequently, its therapeutic effect[18,19]

Here, our aim is to enlarge and deepen the results obtained in the only clinical trial carried out so far[19], evaluating the effect of MI and alpha-LA treatment in a group of Mexican patients affected by PCOS in direct comparison with a group of Italian PCOS women.

Patients and Methods

This is an open-label non-randomized clinical study. A total of 67 patients (26 in Mexico and 41 in Italy) were assessed for eligibility from June 15th, 2019 to July 31st, 2019 at the Human Reproduction Biology Service, Hospital Juarez de México, Avenida Instituto Politécnico Nacional 5160, 07760 Ciudad de México, México, and AGUNCO Medical Center, Viale Sacco e Vanzetti 78, 00155 Rome, Italy.

We applied the following criteria:

Inclusion criteria:
- Patients belonging to the Mexican population.
- Patients belonging to the Italian population.
- Patients between 18 and 40 years old, with anovulation and infertility > 1 year.
- PCOS diagnosis based on the Rotterdam ESHRE-ASRM consensus workshop group[1].
- Presence of IR diagnosed by Homeostatic Model Assessment (HOMA)-Index.

Exclusion criteria:
- Body mass index (BMI) ≥ 30;
- Other conditions causing ovulatory disorders and/or androgen hyper production such as: hyperprolactinemia, hypothyroidism, adrenal hyperplasia, and Cushing syndrome;
- Hormonal and/or pharmacological treatments in the previous 3 months that could interfere with ovulation;
- Drastic changes in diet and physical activity;
- Treatment with products containing inositols in the previous 3 months.

Before entering the study, a written informed consent was obtained from all patients.

This study was performed in line with the principles of the Declaration of Helsinki.

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Study registration number NCT04645745 of the ClinicalTrials.gov registry.

Treatment

In both arms, the enrolled patients were treated orally with 2 g MI, 50 mg alpha-LA, and 200 μg of folic acid twice a day for 6 months. Controls were the same patients at t0 (baseline). The administered compound is in powder, contained in sachets. It must be taken at least 30 minutes before meal.

Primary Outcome

– Evaluation of HOMA-index decrease after 3 and 6 months of MI treatment by monitoring glucose and insulin levels at the enrolment (t0) and at the end.

Secondary Outcomes

– Evaluation of MI treatment in inducing ovulation and reducing hyperandrogenism by controlling BMI and the levels of progesterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), total testosterone, free testosterone, androstenedione, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides. All these parameters were evaluated at baseline (t0) and after 3 (t3) and 6 months (t6) of treatment, except for progesterone which was dosed every month until the end of the study.

Assays in Plasma

The analyses were performed using commercial kits. Glycemic and insulin levels were assessed using the hexokinase G-6-PDH method (Beckman Coulter Diagnostics, Brea, CA, USA) and an enzyme-linked immunosorbent assay (RayBiotech, Inc, Peachtree Corners, GA, USA), respectively. The presence of IR was established by means of the HOMA index. LH and FSH were quantified with enzyme-linked immunosorbent assays (GenWay Biotech. Inc, San Diego, CA, USA). Total cholesterol was measured with an enzymatic cholesterol oxidase/peroxidase method (Beckman Coulter Diagnostics, Brea, CA, USA), and triglycerides were measured with an enzymatic assay (Beckman Coulter Diagnostics, Brea, CA, USA); HDL-cholesterol test and LDL-cholesterol test were performed (Beckman Coulter Diagnostics, Brea, CA, USA); progesterone and total testosterone with an ECLIA (electrochemiluminescence immunoassay) kit (Roche Diagnostics, Mannheim, Germany). Free testosterone and androstenedione were measured with RIA kit (Beckman Coulter Diagnostics, Brea, CA, USA).

Statistical Analysis

Descriptive statistics summarizing quantitative variables included median, 25th and 75th percentiles. Wilcoxon-Mann-Whitney test was used to compare quantitative variables between the two treated groups, while Wilcoxon signed rank sum test was performed to compare the changes from baseline at each time-point in both arms. Data are presented using box plots. Statistical analysis was implemented at two-sided with a 0.05 significance level, using SAS® version 9.4 (SAS Institute Inc. 100 SAS Campus Drive Cary, NC, USA) and Stata™ version 8.2 (StataCorp LLC, College Station, TX, USA).

Results

According to the inclusion and exclusion criteria, a total of 37 anovulatory PCOS women were enrolled in the two arms of this clinical trial.

After the evaluation of 26 PCOS Mexican women, 16 patients were included in the Hospital Juarez de México (Ciudad de México, Mexico), and 14 completed the study, since two patients withdrew during the first month of treatment, due to the beginning of pregnancy. After the evaluation of 41 PCOS Italian women, 21 patients were included in the AGUNCO Medical Center (Rome, Italy); the only dropout, due to personal reasons, occurred in the second week of treatment, whereas the other twenty patients finished the study. Therefore, a total of 34 PCOS women concluded the study. The baseline (t0) age (median) of the two groups patients did not differ significantly (data not shown). Median values of BMI, progesterone, insulin, glucose, HOMA-index, LH, free testosterone, and androstenedione, at baseline were found significantly different between the Mexican and Italian groups (Table I). BMI, progesterone, insulin, HOMA-index, and androstenedione showed higher values at baseline in the Mexican patients compared to the Italian ones, whereas the contrary was found for glucose, LH, and free testosterone (Table I).

The improvement was general, and its extent was more relevant when the starting point was further away from the normal range (it mainly occurred with insulin, HOMA-index, LH, and androstenedione).

In consideration of such differences, the statistical analysis, aimed at comparing monthly or quarterly (depending on the parameter) profiles

Table I. Baseline values of the Italian and Mexican PCOS patients.

Parameter investigated	Italy (n = 20)			Mexico (n = 14)			Mann-Whitney *p*-value
	50th pctl	25th pctl	75th pctl	50th pctl	25th pctl	75th pctl	
BMI t0	25.60	22.80	28.45	28.10	26.30	29.70	0.0099
Progesterone t0	0.60	0.55	0.64	1.15	0.80	1.50	< 0.0001
Insulin t0	18.35	16.95	19.70	25.00	22.00	28.40	< 0.0001
Glucose t0	105.55	101.15	113.70	93.00	88.00	94.00	0.0001
HOMA index t0	4.85	4.40	5.25	5.65	5.20	6.40	0.0043
LH t0	11.90	9.15	13.75	6.45	5.40	9.20	0.0001
FSH t0	5.20	4.50	5.70	5.75	4.50	7.50	0.1610
Total Testosterone t0	91.05	75.50	111.80	92.50	79.00	98.00	0.8063
Free Testosterone t0	1.90	1.75	2.10	1.35	1.10	1.70	0.0004
Androstenedione t0	202.05	160.80	239.60	261.00	220.00	290.00	0.0029
Total cholesterol t0	220.95	209.90	236.60	203.50	198.00	230.00	0.1615
HDL t0	40.05	38.40	48.60	42.50	39.00	52.00	0.5636
LDL t0	144.85	117.00	157.50	131.00	122.00	144.00	0.2015
Triglycerides t0	205.90	187.60	243.20	187.50	113.00	246.00	0.4731

Comparison between the baseline values of the two groups was performed by the Wilcoxon-Mann-Whitney test. *Abbreviations*: BMI: body mass index; HOMA index: Homeostatic Model Assessment; LH: luteinizing hormone; FSH: follicle-stimulating hormone; HDL high-density lipoprotein; LDL low-density lipoprotein; pctl: percentile.

of these patients under treatment, was performed working on the median of variations *vs.* t0, and not on the absolute values.

The median values of BMI (Figure 1) improved significantly after 3 months of treatment in both groups of patients, and the effects were kept until the end of the treatment. BMI was very close to the normality in the Italian group, whereas it was clearly typical of overweight subjects in the case of Mexicans. Its value decreased slightly but significantly in both groups; however, the Mexican patients did not reach the appropriate weight.

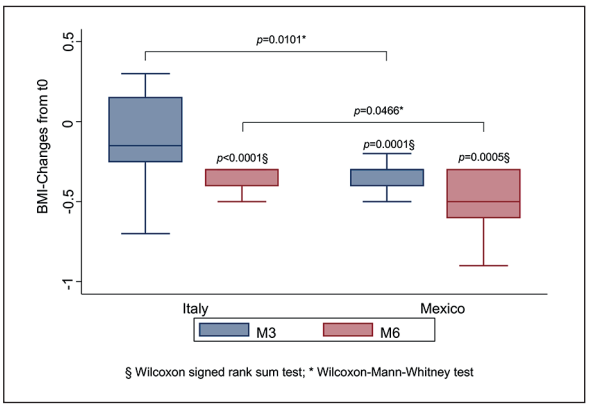

Figure 1. Effect of myo-inositol (MI), alpha-lactalbumin (alpha-LA) and folic acid treatment on body mass index (BMI) variations (medians) in the Italian and Mexican PCOS patients after 3 and 6 months. Comparison between groups was performed using the Wilcoxon-Mann-Whitney test, while change from t0 in each group was analyzed using the Wilcoxon signed rank sum test. Data are presented as box plots. Symbol explanation: the two "whiskers" show the minimum value and maximum value; the rectangle is given by the values between the 25th and 75th percentile; the line inside the rectangle is the median.

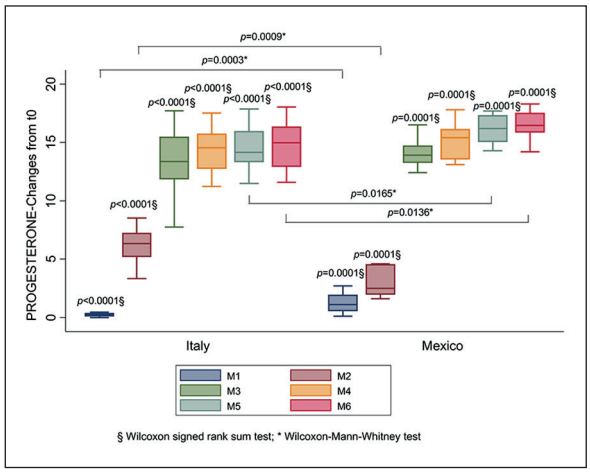

Figure 2. Effect of myo-inositol (MI), alpha-lactalbumin (alpha-LA) and folic acid treatment on progesterone variations (medians) recorded monthly (M1→ M6) in the Italian and Mexican PCOS patients. Comparison between groups was performed using the Wilcoxon-Mann-Whitney test, while change from t0 in each group was analyzed using the Wilcoxon signed rank sum test. Data are presented as box plots. Symbol explanation: the two "whiskers" show the minimum value and maximum value; the rectangle is given by the values between the 25th and 75th percentile; the line inside the rectangle is the median.

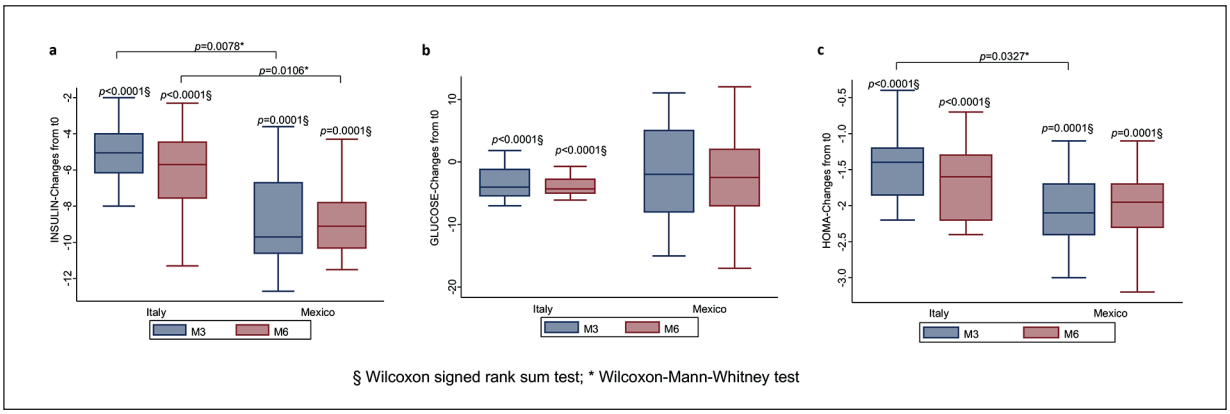

Figure 3. Effect of myo-inositol (MI), alpha-lactalbumin (alpha-LA) and folic acid treatment on insulin **(a)**, glucose **(b)** and Homeostatic Model Assessment (HOMA) index **(c)** variations (medians) in the Italian and Mexican PCOS patients recorded after 3 and 6 months. Comparison between groups was performed using the Wilcoxon-Mann-Whitney test, while change from t0 in each group was analyzed using the Wilcoxon signed rank sum test Data are presented as box plots. Symbol explanation: the two "whiskers" show the minimum value and maximum value; the rectangle is given by the values between the 25th and 75th percentile; the line inside the rectangle is the median.

Progesterone levels, detected every month to check ovulation, significantly increased in each control, reaching satisfactory levels after 3 months of treatment, kept also at 6 months (Figure 2).

Insulin (Figure 3a), glucose (Figure 3b), HOMA-index (Figure 3c) were measured at 3 and 6 months of treatment. All these parameters improved significantly, except for blood sugar in the Mexican women, after 3 months of treatment and the effects were kept until the end of the treatment.

LH (Figure 4a) and FSH (Figure 4b) were also measured at 3 and 6 months of treatment. The most part of these parameters improved, instead, no significant changes were found for FSH in the Italian and for LH in the Mexican patients.

Total testosterone (Figure 5a), free testosterone (Figure 5b) and androstenedione (Figure 5c) showed significant reductions already at 3 months. Moreover, androstenedione decreased significantly also at 6 months versus 3 months.

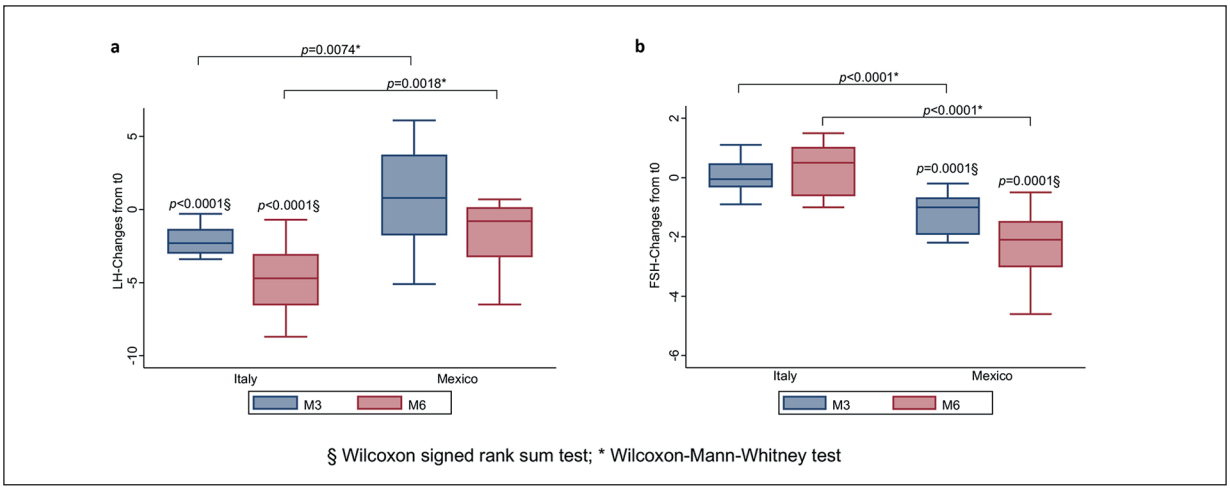

Figure 4. Effect of myo-inositol (MI), alpha-lactalbumin (alpha-LA) and folic acid treatment on Luteinizing hormone (LH) **(a)** and Follicle-stimulating hormone (FSH) **(b)** variations (medians) in the Italian and Mexican PCOS patients recorded after 3 and 6 months. Comparison between groups was performed using the Wilcoxon-Mann-Whitney test, while change from t0 in each group was analyzed using the Wilcoxon signed rank sum test. Data are presented as box plots. Symbol explanation: the two "whiskers" show the minimum value and maximum value; the rectangle is given by the values between the 25th and 75th percentile; the line inside the rectangle is the median.

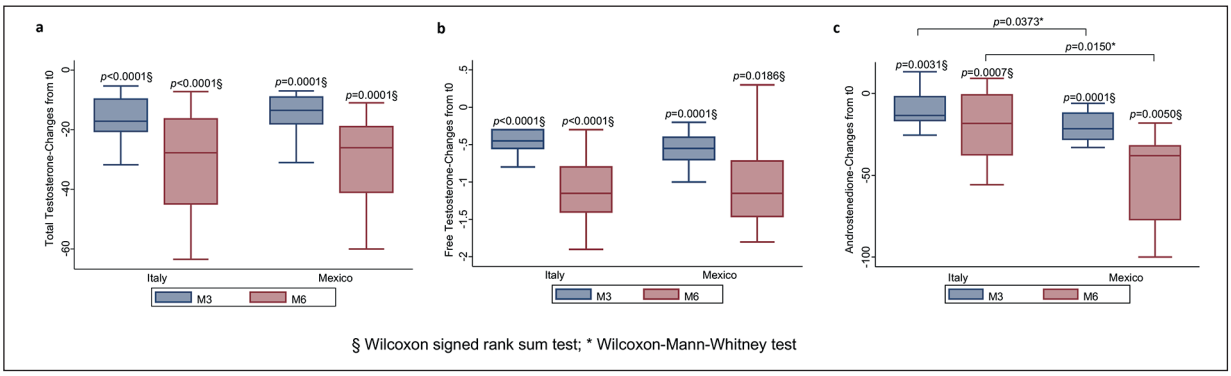

Figure 5. Effect of myo-inositol (MI), alpha-lactalbumin (alpha-LA) and folic acid treatment on total testosterone **(a)**, free testosterone **(b)** and androstenedione **(c)** variations (medians) in the Italian and Mexican PCOS patients recorded after 3 and 6 months. Comparison between groups was performed using the Wilcoxon-Mann-Whitney test, while change from t0 in each group was analyzed using the Wilcoxon signed rank sum test. Data are presented as box plots. Symbol explanation: the two "whiskers" show the minimum value and maximum value; the rectangle is given by the values between the 25th and 75th percentile; the line inside the rectangle is the median.

Total cholesterol (Figure 6a), LDL (Figure 6c) and triglycerides (Figure 6d) improved significantly after 3 months of treatment and the effects were kept until the end of the treatment. No significant changes were found for HDL (Figure 6b) in the Italian and in the Mexican group.

Adverse effects were very few and of little importance or even none and affected only the Mexican women where two patients experienced occasional episodes of nausea/vomiting or abdominal colic.

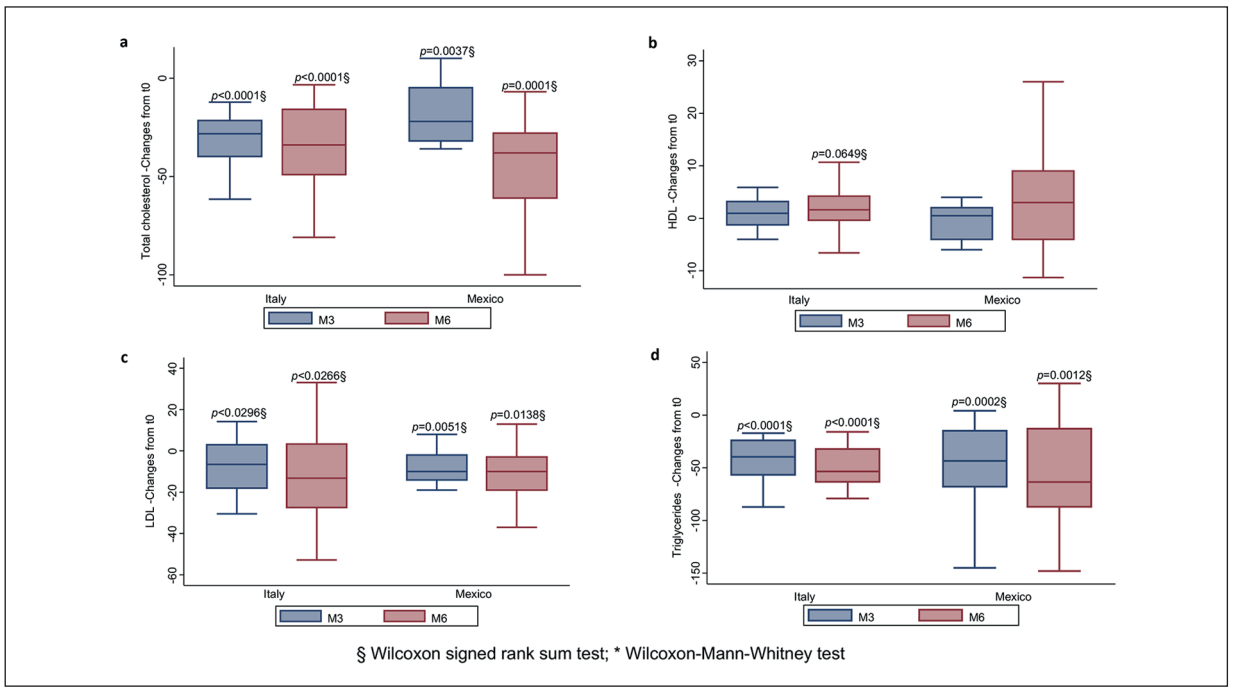

Figure 6. Effect of myo-inositol (MI), alpha-lactalbumin (alpha-LA) and folic acid treatment on total cholesterol **(a)**, high-density lipoprotein (HDL) **(b)**, low density lipoprotein (LDL) **(c),** and triglycerides **(d)** variations (medians) in the Italian and Mexican PCOS patients recorded after 3 and 6 months. Comparison between groups was performed using the Wilcoxon-Mann-Whitney test, while change from t0 in each group was analyzed using the Wilcoxon signed rank sum test. Data are presented as box plots. Symbol explanation: the two "whiskers" show the minimum value and maximum value; the rectangle is given by the values between the 25th and 75th percentile; the line inside the rectangle is the median.

Discussion

We confirmed that MI and alpha-LA treatment can significantly improve several parameters. Our clinical trial gives a strong support to the administration of MI plus alpha-LA to overweight PCOS women based on the results obtained in two very different populations, Italians and Mexicans with different BMI and IR values. Previous studies showed that the prevalence of PCOS in a group of Mexican women residing in Mexico City is between 6.0% (according to NIH criteria) and 6.6% (according to Rotterdam criteria)[20]. It is a low value if compared to higher percentages found in other populations (up to 20%)[21]. Of note, such a percentage differs from that reported in a population of Mexican women living in USA[22], where it increases about twofold, reaching 12.8%, in part explainable by means of the different diets in Mexico and USA. An increase in adiposity[23] is a typical feature of Mexican Americans. Furthermore, the females of this population are characterized by higher centrality indices and waist-to-hip ratios (WHRs) than non-Hispanic white counterpart, even when these parameters are adjusted for demography (age, menopausal status) and behavior (BMI, parity, smoking, alcohol use, physical exercise, oral contraceptive and estrogen administration). No correlation was found between body fat distribution and behavioral variables and this evidence supports the involvement of genetic factors in the distribution of body fat in this population, regardless of environmental factors[24]. Mexican women also suffer from increased prevalence of hyperinsulinemia[25], IR, and metabolic syndrome. IR shows higher levels among them also when compared to non-Hispanic whites and blacks[26]. A large body of scientific evidence sustains that the Mexican population presents a genetic predisposition to metabolic syndrome, type 2 diabetes, as well as many forms of dyslipidemia[27]. In our sample, all patients were strongly overweight (without lean women) as well as insulin-resistant, as expected since IR rate is higher in overweight and obese women than in normal-weight women. Therefore, this group of patients is representative of the PCOS Mexican population with BMI over the normal value. Hence, the baseline difference between the Mexican and Italian subjects found in our study may be explained by the specific genetic, nutritional, and behavioral profile of the two populations.

Overweight or obesity are associated with a systemic chronic inflammation of low-grade that can contribute to the onset of IR[28]. Of note, an increase in the circulating levels of some inflammatory biomarkers (Tumor Necrosis Factor, Interleukin 6 and 18, monocyte chemoattractant protein-1, C-reactive protein, count of lymphocytes and monocytes) was detected in PCOS women[29]. This demonstrates that mild or moderate inflammation, perhaps not affecting all patients, is also a feature of this syndrome. The link between inflammation and PCOS is very complex and, so far, not completely understood, with the involvement of other factors besides those already mentioned. We refer to external factors which are among the possible causes of PCOS onset, that is the altered microbiota[30], displaying an inflammatory profile. The resultant chronic activation of hepatic and tissue macrophages causes impaired insulin receptor function with the consequent onset of IR. The state of hyperinsulinemia interferes with the normal follicle development in the ovary, causing menstrual irregularity. High serum insulin also drives excessive androgen production by ovarian, resulting in a net increase in free androgen availability and the development of acne and hirsutism[30]. Dysbiosis, chronic inflammation and the associated consequences are more pronounced in obese PCOS women[31]. It suggests that modulation of gut microbiota may be a potential way for the treatment of PCOS[32]. Of note, dysbiosis appears to affect also the absorption of some nutrients[33].

In this landscape of harmful changes, alpha-LA can exert some important beneficial effects, both as an intestinal absorption enhancer and as molecule able to directly reduce some PCOS-related disorders, in such a way integrating and strengthening the MI therapeutic activity.

Conclusions

In summary, it is highly plausible that the advantageous changes in the composition of gut microbiota due to the prolonged administration of alpha-LA can improve MI intestinal absorption. Indeed, it was demonstrated that dietary administration of alpha-LA can positively influence gut microbiota[34]. Furthermore, alpha-LA is an anti-inflammatory molecule, endowed with the capacity to inhibit type 2 cyclooxygenase (COX 2) and to decrease the inflammatory cytokine IL-

6[35]. In addition, alpha-LA was able to decrease blood glucose levels after glucose loading in a rat model of type 2 diabetes[36]. Therefore, alpha-LA per se can strongly reduce the chronic inflammation connected to PCOS, also with reference to IR, thus cooperating with the healing activity due to MI. Such an effect contributes to explain the results of our study also in the Mexican women, characterized by an increased BMI and a more relevant IR in comparison to the Italian patients.

Conflict of Interest

Vittorio Unfer is an employee at Lo.Li. Pharma s.r.l., Rome, Italy. The other authors have no conflicts of interest associated with this clinical trial.

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