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ORIGINAL PAPER / GYNECOLOGY

Effect of 40:1 myo-inositol/D-chiro-inositol treatment on serum asprosin levels in polycystic ovary syndrome: a prospective randomized pilot study

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ABSTRACT

Objectives: Asprosin, a novel adipokine primarily secreted by white adipose tissue, has been implicated in the pathogenesis of insulin resistance and metabolic dysfunction. Its elevated levels characterize patients with polycystic ovary syndrome (PCOS) exhibiting metabolic alterations. Aim of this work was to evaluate the effects of insulin sensitizer administration, as inositols, on asprosin levels and then compare these results with the effects of metformin treatment.

Material and methods: 30 patients with PCOS were enrolled in this study and randomly divided into two groups: (i) group 1 assumed a dietary supplement based on 40:1 myo-inositol (MI)/D-chiro-inositol (DCI), (ii) group 2 assumed metformin (MET), for 12–16 weeks of treatment.

Results: The reduction of serum asprosin levels in patients with PCOS after treatment with MET and MI/DCI is the most intriguing result. Its levels decreased more in the inositol group, although they did not reach statistical significance probably due to the lower number of patients.

Conclusions: The observed dysregulation of asprosin levels in PCOS highlights a potential link between this novel adipokine and the pathophysiology of the disorder, suggesting a modulatory effect of the combined 40:1 MI/DCI on asprosin levels. Of course, further studies may contribute to disclosing molecular mechanisms underlying asprosin reduction and open toward new perspectives.

Keywords: asprosin; PCOS; myo-inositol; D-chiro-inositol; α-lactalbumin

INTRODUCTION

Asprosin was first identified in 2016, and it has been shown to regulate hepatic glucose release [1]. Asprosin is the C-terminal product of profibrillin and white adipose tissue (WAT) represents its major source [1]. Since the discovery of asprosin, numerous articles have been published about its effect on glucose and insulin metabolism, while previous publications reported that asprosin can also cross blood-brain barrier and act as an orexigenic hormone [2]. Studies in literature suggest that it positively correlates with obesity and insulin resistance (IR), indeed its levels are elevated in patients with type 2 diabetes mellitus (T2DM)

[3–5]. Evidence indicates that it may play a role in the pathogenesis of metabolic syndrome, and it may be a new biomarker for obesity and metabolic syndrome [6, 7]. In addition, asprosin has been shown to affect insulin metabolism as well as inflammatory processes [8]. Polycystic ovary syndrome (PCOS) is a highly prevalent endocrinological disorder of reproductive age [9]. It is characterized by ovarian dysfunction, hyperandrogenism, polycystic ovarian morphology, and it is associated with increased risk of developing cardiac disease, T2DM, and metabolic syndrome in advanced age [10, 11]. Although it is still not among the diagnostic criteria, glucose metabolism impairment, hyperinsulinemia and hyperglycemia are frequently observed among patients with PCOS, thus indicating that insulin may have a role in the pathogenesis of this syndrome [10]. In line with this, recent publications from the Experts Group on Inositol in Basic and Clinical Research, and on PCOS (EGOI-PCOS) have started to stimulate a new reading of Rotterdam PCOS criteria proposing a focus on hyperandrogenism and hyperinsulinemia. In particular, the EGOI-PCOS proposal differentiates between three phenotypes of PCOS characterized by metabolic and endocrine alterations (ex-phenotypes A–C of Rotterdam criteria) and one phenotype (ex-phenotype D of Rotterdam criteria) resembling a clinical picture of PCOS with only ovarian and menstrual alterations, without hyperandrogenism or IR [12–14]. More in detail, the EGOI-PCOS considers hyperandrogenism as consequence of metabolic alterations, more specifically of IR, thus proposing a new set of PCOS diagnostic criteria that identify the ex-phenotypes A–C of Rotterdam criteria as an endocrine metabolic syndrome (EMS) type 1–3 according to different criteria [15–17]. This is in line with a recent work by de Zegher and Ibáñez [18] focusing on the leading role of IR in the pathogenesis of PCOS compared to hyperandrogenism.

In PCOS affected patients, serum asprosin levels are higher than healthy women and they correlated with IR [19]. Similar results were found in a recent meta-analysis that compared serum asprosin levels of 1050 PCOS patients with 796 healthy women [20]. These data suggest that asprosin may play a role in impaired glucose metabolism observed in patients with PCOS exhibiting metabolic alterations.

When impaired glucose metabolism occurs in PCOS condition, insulin sensitizers like metformin (MET) and inositol derivatives, are commonly used in the management of the syndrome. Of these myo-inositol (MI) and D-chiro inositol (DCI) are two of nine stereoisomers of the inositol family, and they act as secondary messengers of the insulin signaling pathway, facilitating the translocation of glucose transporters to the cell membrane and promoting glucose uptake, respectively [21]. Studies suggest that an imbalance in the

ratio of MI to DCI may contribute to IR, thus exploring the therapeutic potential role of inositol supplementation in improving insulin sensitivity and mitigating metabolic dysfunction [21]. Previous clinical studies reported that 40:1 MI/DCI is the best ratio to recover ovulation [22] and metabolic and hormonal balance in PCOS affected patients with metabolic alterations [23–25].

Furthermore, evidence in literature demonstrated that adding a prebiotic molecule, as the α-lactalbumin (α-LA), to inositol formulation improves their intestinal absorption [26] and consequently their clinical effectiveness [27–29]. As insulin-sensitizers, inositols (both MI and DCI) and MET have similar effects on improving insulin resistance in patients with PCOS, and additionally, both of them affect WAT/BAT (brown adipose tissue) metabolism [30–33]. Considering together the relationship of asprosin with glucose metabolism and the impaired glucose metabolism observed in PCOS condition, it can be reasonable that insulin sensitizing drugs, such as inositol or MET, may improve serum asprosin levels.

Objectives

The aim of the study was to determine the effect of the dietary supplement based on 40:1 MI/DCI plus α-LA on serum asprosin levels and then compare results with the effects of MET treatment.

MATERIAL AND METHODS

This prospective randomized study was conducted at Near East University Hospital between 1.09.2021–3.06.2023. Ethical approval was obtained from Near East University Ethics Review Board with the number of YDU/2020/76-993. The study was registered at clinicaltrials.gov (NCT05951309). Informed written consent was obtained from all participants.

The study population consisted of 30 patients with PCOS diagnosed according to Rotterdam criteria between the ages of 18–35 [10]. Demographic characteristics (age, weight, height, gravida, parity), Ferriman–Gallwey Scoring (FGS), and ultrasonography examination findings of the patients who volunteered to participate in the study, were recorded at the baseline between the 2^{nd}–3^{rd} day of menstruation. Then, follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol, prolactin (PRL), thyroid stimulating hormone (TSH), androstenedione, sex hormone binding globulin (SHBG), dehydroepiandrosterone sulfate (DHEAS), total testosterone (TT), free testosterone (fT), fasting glucose (fG) and fasting

insulin tests were performed using commercial immunoassays with morning fasting blood. One tube of blood was taken from the patients and centrifuged, the separated serum part was kept at −80°C for serum asprosin analysis. Afterwards, the patients were randomized according to the application protocol number: group 1 took 550 mg MI + 13.8 mg DCI + 14.1 mg α-LA + 200 µg folic acid (Inofolic combi HP, Lo.Li. pharma s.r.l. Rome, Italy) twice a day, while group 2 took 500 mg MET (Glucophage, Merck, Istanbul, Turkiye) twice a day. Enrollment in the study was finalized when both groups reached 15 participants. The treatment has lasted 12–16 weeks of treatment, in accordance with a previous work by Nordio et al. [22], and after the treatment period all patients were checked again on the 2nd or 3rd day of menstruation. Evaluations at the first application were repeated and one tube of blood was centrifuged and stored for asprosin testing. In patients with menstrual irregularities, menstruation that was achieved with tarlusal after pregnancy was excluded (5 mg medroxyprogesterone acetate twice a day; Tarlusal; Deva Holding A.S., Istanbul, Turkiye).

Serum asprosin test was performed by a sandwich enzyme-linked immunosorbent assay kit (USCN Life Science Inc., Wuhan, China Catalog no. SEA332Hu). For evaluating insulin resistance, the homeostasis model assessment-insulin resistance (HOMA-IR) was used. HOMA-IR was calculated by multiplying fasting blood glucose and fasting insulin levels and dividing by 405, whereas the body mass index (BMI) was calculated based on the ratio of weight to height in square meters. Free androgen index (FAI) was calculated by the formula: FAI = 100× (TT/SHBG). HOMA-IR, BMI, and FAI were calculated both at the first examination and after 12–16 weeks of treatment.

Exclusion criteria were the occurrence of chronic hypertension, diabetes, pregnancy, assumption of any insulin sensitizers before the enrollment, addiction to substances such as smoking or alcohol, having thyroid or other endocrine disorders.

RESULTS

The study analyzed the results from a total of 30 patients affected by PCOS. The two groups were similar in terms of demographic characteristics. The laboratory parameters at the first examination (T0) and the 2nd examination (T1) of the treatment are shown in Table 1. At baseline, serum levels of FSH were statistically significantly higher in group 2 ($p = 0.023$), although not significant clinically. The other blood test results were similar for both groups (Tab. 1).

Table 2 shows changes in parameters from the initial examination (before starting the treatment) until the end of treatment within the group. In group 1, BMI, FGS, serum asprosin, androstenedione, DHEAS, TT, fT, fG, insulin, LH, HOMA-IR, FAI values significantly decreased after treatment. In group 2, the decrease in FGS, asprosin, androstenedione, TT, fG, insulin, HOMA-IR, and FAI were statistically significant. However, by evaluating the groups in terms of variation of the different parameters, only serum levels of fT statistically decreased in group 1 compared to group 2 ($p = 0.003$) (Tab. 3).

DISCUSSION

The observed dysregulation of asprosin levels in the context of PCOS highlights a potential link between this novel adipokine and the pathophysiology of the disorder. Asprosin, primarily secreted by WAT, has been implicated in the pathogenesis of IR and metabolic dysfunction [1]. The most intriguing result of this study is that serum asprosin levels, which are high in PCOS condition, decrease after treatment with MET or 40:1 MI/DCI treatment, which are insulin sensitizing agents.

In literature the role of MI and DCI was highlighted in various cellular processes, including insulin signaling pathways and glucose metabolism [34]. According to studies by Croze et al. [30] and Monastra et al. [31], inositol may exert modulatory effects on WAT, influencing adipocyte function and potentially contributing to metabolic homeostasis. The observed interactions between MI/DCI and WAT in literature shed light on a potentially significant aspect of metabolic regulation [30, 31]. The interplay between asprosin and inositol observed in our study unveils a potentially intricate relationship at the intersection of novel adipokines and cellular signaling pathways. Also, our findings suggest a potential modulatory effect of the combined 40:1 MI/DCI on asprosin levels, adding a layer of complexity to the understanding of adipokine dynamics. In addition, our study contributes to this body of knowledge by proposing for the first time a connection between inositol and the regulation of asprosin in PCOS affected women exhibiting metabolic alterations. While the precise mechanisms underlying inositol’s effects on insulin and glucose metabolism are complex and multifaceted, their involvement in cellular processes highlights their importance in maintaining metabolic homeostasis. The effect of MI and DCI in inducing WAT/BAT trans differentiation may be the reason for the decrease in serum levels of asprosin, which is indeed produced from WAT.

Furthermore, the presence of α-LA in the administered dietary supplement guarantees a higher intestinal absorption and clinical effectiveness in patients with PCOS [27–29]. Indeed, patients with metabolic alterations and elevated BMI may be characterized by intestinal inflammation and dysbiosis of gut microbiota that could reduce micronutrient absorption. Therefore, the prebiotic activity of α-LA may recover gut dysbiosis condition and improve clinical outcomes in PCOS condition [35].

Yuan et al. [32] observed the effects of MET on WAT in their study, and they found that MET has an effect on the regulation of some proteins of WAT and BAT. However, the impact of MET on WAT extends beyond its well-established role in improving insulin sensitivity [36]: a recent study showed that MET has a decreasing effect on blood asprosin levels [37]. Therefore, considering that our findings are in accordance with previous data in literature, we can conclude that MET can modulate adipose tissue function and affect adipokine secretion and adipocyte metabolism.

This study examined for the first time the effects of MET and 40:1 MI/DCI treatments on serum asprosin levels in women with PCOS. Although such levels decreased more in the inositol group compared to the MET group, this result did not reach statistical significance. However, this may be due to the relatively small number of patients, therefore a study with more participants could help to determine the superiority of 40:1 MI/DCI compared to MET in reducing serum asprosin levels in patients with PCOS with metabolic impairments. In addition, molecular mechanisms through which MET and MI/DCI induce the asprosin-reducing effect remain still unclear and further research may open toward new perspectives.

The relationship of inositol and MET with asprosin and WAT warrants further investigation into the specific involved molecular pathways, offering potential avenues for therapeutic interventions aimed at managing metabolic disorders. Future studies may explore the impact of MI and DCI supplementation on adipokine secretion and adipocyte phenotype, providing a more comprehensive understanding of their role in WAT function.

Evidence in literature reports that both inositol and MET have improved effects on serum androgens and glucose metabolism [33, 38, 39]. Our study showed that fasting glucose, fasting insulin, insulin resistance, and some androgen levels including the FAI index, improved in both groups, consistent with previous evidence, and corroborating the beneficial effects of insulin-sensitizer agents in patients with PCOS with metabolic-endocrine alterations. Only BMI, DHEAS, fT, and LH levels significantly decreased in group 1 with 40:1 MI/DCI treatment, while no significant difference occurred in group 2.

In this study, by comparing changes before and after treatment within the group, more parameters improved under 40:1 MI/DCI treatment rather than under MET. However, when these change percentages were compared between groups, only the fT level showed a significant decrease in group 1.

Considering the positive effects on metabolic and androgen profile, these data result in accordance with previous evidence by Unfer and colleagues, thus corroborating the importance of using specific approaches depending on the phenotype of patients with PCOS [40]. Overall, based on these results, we can conclude that 40:1 MI/DCI formulation seems to be more effective than MET in some cases in patients with PCOS exhibiting metabolic and endocrine alterations.

In addition, it is crucial to mention the safety of inositol treatment, indeed at clinical dosage of 4 grams daily is completely free of side effects [41]. Even though clinical study is a randomized controlled study, the small number of participants, correlated to the design of the pilot study, may be a critical limitation to the observed results. For this reason, larger clinical studies also considering patients with different phenotypes of PCOS according to the new set of EGOI-PCOS diagnostic criteria [15], will help to corroborate these data and to study levels of asprosin among the different phenotypes.

CONCLUSIONS

In conclusion, both 40:1 MI/DCI and MET decrease serum asprosin levels in women with PCOS, also confirming asprosin as a new possible biomarker for IR condition. Considering the central role of adipose tissue in metabolic homeostasis, the potential of MI/DCI and MET to target WAT is particularly important for the possible impact on asprosin levels. The results of this study lay the groundwork for expanding evidence with a more in-depth understanding of asprosin levels in different phenotypes of PCOS.

Article information and declarations

Data availability statement

Data will be made available upon reasonable request.

Ethics statement

Ethical approval was obtained from Near East University Ethics Review Board with the number of YDU/2020/76-993. The study was registered at clinicaltrials.gov (NCT05951309). Informed written consent was obtained from all participants.

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None.

Author contributions

Conceptualization — ACO; methodology — YO, OE; investigation — OEO, YO, OE, ACO; writing: original draft preparation — OEO and ACO; writing: review and editing — MC, YO and OE; supervision — ACO. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare no conflicts of interest.

Supplementary material

None.

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Table 1. Comparison of demographic characteristics and laboratory findings of groups

	Group 1 (n = 15)	**Group 2 (n = 15)**	**p value***
Age [years]	23.7 ± 2.8	24.5 ± 2.8	0.395
BMI T0 [kg/m^2]	27.15 ± 4.63	27.62 ± 4.51	0.741
BMI T1 [kg/m^2]	26.63 ± 3.95	26.97 ± 3.48	0.682
FGS T0	11.07 ± 2.84	11.2 ± 2.65	0.920
FGS T1	9.6 ± 2.2	10.07 ± 2.55	0.646
ASP T0 [ng/mL]	36.11 ± 22.119	39.056 ± 20.726	0.711
ASP T1 [ng/mL]	25.103 ± 14.426	34.887 ± 18.928	0.105
Androstenedione T0 [ng/dL]	149.3 ± 49.2	146 ± 48.8	0.453
Androstenedione T1 [ng/dL]	114.3 ± 21.5	129.8 ± 35.4	0.298
DHEAS T0 [µg/dL]	460.6 ± 119.6	486.4 ± 127.3	0.478
DHEAS T1 [µg/dL]	385.3 ± 93.5	443.7 ± 121.6	0.280
TT T0 [nmol/L]	1.92 ± 0.57	1.96 ± 0.61	0.881
TT T1 [nmol/L]	1.48 ± 0.41	1.82 ± 0.57	0.067
SHBG T0 [nmol/L]	34.35 ± 19.05	37.9 ± 18.4	0.562
SHBG T1 [nmol/L]	39.79 ± 15.69	45.7 ± 27.09	1.000
fT T0 [pg/mL]	2.29 ± 0.67	2.01 ± 0.71	0.352
fT T1 [pg/mL]	1.6 ± 0.54	1.97 ± 0.56	0.159
Fasting Glucose T0 [mg/dL]	95.47 ± 6.52	92.87 ± 7.41	0.342
Fasting Glucose T1 [mg/dL]	89.8 ± 5.16	88 ± 5.01	0.395
Insulin T0 [mU/L]	13.69 ± 4.38	12.03 ± 5.95	0.271
Insulin T1 [mU/L]	10.67 ± 3.7	9.65 ± 4.12	0.478
HOMA-IR T0 (ratio)	3.26 ± 1.11	2.82 ± 1.52	0.254
HOMA-IR T1 (ratio)	2.38 ± 0.84	2.11 ± 0.95	0.418
TSH (mU/L)	2.17 ± 1.19	1.68 ± 0.48	0.165
Prolactin [ng/mL]	16.66 ± 6.26	16.71 ± 4.4	0.711
FSH T0 [IU/L]	4.22 ± 0.56	4.78 ± 0.76	**0.023**
FSH T1 [IU/L]	4.61 ± 0.87	4.91 ± 0.81	0.418
LH T0 [IU/L]	5.82 ± 3.49	5.6 ± 2.82	0.904
LH T1 [IU/L]	4.55 ± 1.36	5.51 ± 2.13	0.215
Estradiol T0 [pg/mL]	37.07 ± 17.89	36.33 ± 16.62	1.000
Estradiol T1 [pg/mL]	38.67 ± 8.7	31.87 ± 9.93	0.072
FAI T0 (index)	7.54 ± 5.92	7.03 ± 4.88	0.834
FAI T1 (index)	4.15 ± 1.77	5.22 ± 2.97	0.384

*Mann–Whitney U test; ASP — asprosin; BMI — body mass index; DHEAS — dehydroepiandrosterone sulfate; FAI — free androgen index; FGS — Ferriman–Gallwey score; FSH — follicle stimulating hormone; fT — free

testosterone; HOMA-IR — homeostasis model assessment of insulin resistance; LH — luteinizing hormone; SHBG — sex hormone binding globulin; TSH — thyroid stimulating hormone; TT — total testosterone

Table 2. Comparison of clinical and laboratory findings within groups

	Group 1 (n = 15)			**Group 2 (n = 15)**		
	Before	**After**	**p value***	**Before**	**After**	**p value***
BMI (kg/m^2)	27.15 ± 4.63	26.63 ± 3.95	**0.028**	27.62 ± 4.51	26.97 ± 3.48	0.073
FGS	11.07 ± 2.84	9.6 ± 2.2	**0.003**	11.2 ± 2.65	10.07 ± 2.55	**0.003**
ASP [ng/mL]	36.11 ± 22.109	25.103 ± 14.426	**0.005**	39.056 ± 20.726	34.887 ± 18.928	**0.026**
Androstenedione [ng/dL]	149.3 ± 49.2	114.3 ± 21.5	**0.002**	146 ± 48.8	129.8 ± 35.4	**0.003**
DHEAS [µg/dL]	460.6 ± 119.6	385.3 ± 93.5	**0.003**	486.4 ± 127.3	443.7 ± 121.6	0.069
TT [nmol/L]	1.92 ± 0.57	1.48 ± 0.41	**0.006**	1.96 ± 0.61	1.82 ± 0.57	**0.038**
SHBG [nmol/L]	34.35 ± 19.05	39.79 ± 15.69	0.211	37.9 ± 18.4	45.7 ± 27.09	0.089
fT [pg/mL]	2.29 ± 0.67	1.6 ± 0.54	**0.001**	2.01 ± 0.71	1.97 ± 0.56	0.139
fG [mg/dL]	95.47 ± 6.52	89.8 ± 5.16	**0.005**	92.87 ± 7.41	88 ± 5.01	**0.002**
Insulin [mU/L]	13.69 ± 4.38	10.67 ± 3.7	**0.001**	12.03 ± 5.95	9.65 ± 4.12	**0.004**
HOMA-IR (ratio)	3.26 ± 1.11	2.38 ± 0.84	**0.001**	2.82 ± 1.52	2.11 ± 0.95	**0.002**
FSH [IU/L]	4.22 ± 0.56	4.61 ± 0.87	0.078	4.78 ± 0.76	4.91 ± 0.81	0.497
LH [IU/L]	5.82 ± 3.49	4.55 ± 1.36	**0.031**	5.6 ± 2.82	5.51 ± 2.13	0.912
Estradiol [pg/mL]	37.07 ± 17.89	38.67 ± 8.7	0.363	36.33 ± 16.62	31.87 ± 9.93	0.246
FAI (index)	7.54 ± 5.92	4.15 ± 1.77	**0.023**	7.03 ± 4.88	5.22 ± 2.97	**0.026**

*Wilcoxon test; ASP — asprosin; BMI — body mass index; DHEAS — dehydroepiandrosterone sulfate; FAI — free androgen index; fG — fasting glucose; FGS — Ferriman–Gallwey score; FSH — follicle stimulating hormone; fT — free testosterone; HOMA-IR — homeostasis model assessment of insulin resistance; LH — luteinizing hormone; SHBG — sex hormone binding globulin; TT — total testosterone

Table 3. Change of the laboratory findings of participants

	ΔMI/DCI	**ΔMET**	**p value***
BMI	−0.521 ± 1.222	−0.647 ± 1.222	0.967
FGS	−1.467 ± 1.302	−1.133 ± 0.915	0.595
ASP	−11.007 ± 13.460	−4.170 ± 7.221	0.233
Androstenedione	−34.98 ± 33.00	−16.19 ± 21.62	0.067
DHEAS	−75.29 ± 83.77	−42.67 ± 85.74	0.389
TT	−0.439 ± 0.531	−0.137 ± 0.269	0.116
SHBG	5.441 ± 15.198	7.800 ± 22.256	0.713
fT	−0.684 ± 0.542	−0.040 ± 0.504	**0.003**
Fasting glucose	−5.67 ± 5.95	−4.87 ± 4.56	0.775
Insulin	−3.01 ± 2.70	−2.39 ± 2.51	0.512
HOMA-IR	−0.883 ± 0.664	−0.705 ± 0.674	0.539
FSH	0.391 ± 0.919	0.131 ± 0.991	0.744
LH	−1.270 ± 2.315	−0.927 ± 2.265	0.174
Estradiol	1.600 ± 15.806	−4.467 ± 12.733	0.106
FAI	−3.393 ± 5.838	−1.808 ± 3.081	0.539

*Mann-Whitney U test; ASP — asprosin; BMI — body mass index; DHEAS — dehydroepiandrosterone sulfate; FAI — free androgen index; FGS — Ferriman–Gallwey score; FSH — follicle stimulating hormone; fT — free testosterone; ; HOMA-IR — homeostasis model assessment of insulin resistance; LH — luteinizing hormone; SHBG — sex hormone binding globulin; TT — total testosterone