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ORIGINAL ARTICLE

Oral myo-inositol plus D-chiro-inositol (3.6:1) reduces insulin resistance pathway biomarkers in acne-involved skin among subjects with metabolic comorbidities: A gene expression study.

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Abstract

BACKGROUND: Hyperinsulinemia and insulin resistance promote acne pathogenesis through reduced Forkhead box protein O1 (*FOXO1*) signaling and increased mechanistic target of rapamycin (*MTOR*) and insulin-like growth factor-1 (*IGF1*) activity. While oral myo-inositol (MI) and D-chiro-inositol (DCI) supplementation may improve insulin sensitivity, evidence regarding direct pathway modulation in acne-involved skin remains limited. In this exploratory, single-arm, uncontrolled study, we sought to investigate the effects of oral MI/DCI (3.6:1 ratio) on cutaneous expression of these biomarkers in adults with acne and metabolic comorbidities (polycystic ovary syndrome or metabolic syndrome).

METHODS: Forty-five adults with active inflammatory acne on the back were enrolled across three subgroups of 15 – including women with polycystic ovary syndrome (PCOS), women with metabolic syndrome (MetS), and men with MetS. All participants received oral MI/DCI (1100 mg + 300 mg daily) for 12 weeks in a single-arm, open-label, pre-post biopsy study. Paired punch biopsies of acne-involved skin were obtained before and after supplementation, and *FOXO1*, *MTOR*, and *IGF1* mRNA expression was quantified by qRT-PCR. Clinical response was assessed by Investigator Global Improvement rating.

RESULTS: Following the 12-week supplementation, *FOXO1* expression increased (Cohen's d = +1.13; $p < 0.001$), whereas *MTOR* (Cohen's $d = -3.46$; $p < 0.001$) and *IGF1* (Cohen's d = −1.62; $p < 0.001$) decreased significantly compared with baseline. Changes were directionally consistent across all subgroups. Clinical improvements were observed in 82.2% of participants. Plasma testosterone in men remained stable ($p = 0.638$).
CONCLUSIONS: In this exploratory, uncontrolled study, 12 weeks of oral MI/DCI supplementation (3.6:1 ratio) was associated with significant changes in insulin resistance pathway gene expression in acne-involved skin. These findings provide preliminary mechanistic support for further randomized, controlled trials evaluating MI/DCI as a potential adjunctive strategy in acne associated with metabolic comorbidities.

Abbreviations:

ANOVA	- analysis of variance
BMI	- body mass index
CI	- confidence interval
DCI	- D-chiro-inositol
FOXO1	- Forkhead box protein O1
GAPDH	- glyceraldehyde-3-phosphate dehydrogenase
HOMA-IR	- homeostatic model assessment of insulin resistance
IGF1	- insulin-like growth factor-1
MetS	- metabolic syndrome
MI	- myo-inositol
MTOR	- mechanistic target of rapamycin
PCOS	- polycystic ovary syndrome
qRT-PCR	- quantitative reverse transcription polymerase chain reaction

INTRODUCTION

Acne vulgaris is a chronic inflammatory dermatosis of the pilosebaceous unit affecting not only adolescents but also a substantial proportion of the adult population (Kutlu *et al.* 2023). Although classical pathogenic models have focused on abnormal follicular keratinization, excess sebum production, and *Cutibacterium acnes* colonization (Kurokawa & Nakase, 2020; Kim & Kim, 2024), metabolic and endocrine abnormalities are increasingly recognized as major contributors to disease development (Pareek *et al.* 2022; Endres *et al.* 2025). Hyperinsulinemia and insulin resistance may promote acne pathogenesis through reduced Forkhead box protein O1 (*FOXO1*) signaling (Kamboj *et al.* 2024) as well as increased mechanistic target of rapamycin (*MTOR*) (Monfrecola *et al.* 2016) and insulin-like growth factor-1 (*IGF1*) activity (Gu *et al.* 2022) – which in turn stimulate sebocyte proliferation and lipogenesis (Fabbrocini *et al.* 2016). The clinical relevance of these metabolic derangements is particularly apparent in polycystic ovary syndrome (PCOS), where acne has been reported as a comorbidity in up to 49% of affected patients (Pourahmad *et al.* 2025), and in metabolic syndrome (MetS), where systemic inflammation and hyperinsulinemia have been associated with treatment-resistant presentations (Chandak *et al.* 2022; Endres *et al.* 2025).

Inositols – particularly the stereoisomers myo-inositol (MI) and D-chiro-inositol (DCI) – serve as essential second messengers in insulin signaling (Bevilacqua & Bizzarri, 2018). In insulin-resistant states, the conversion of MI to DCI is impaired, resulting in localized DCI deficiency (Kalra *et al.* 2016). Oral supplementation with combined MI and DCI has been shown to restore insulin sensitivity and improve clinical manifestations of insulin resistance (DiNicolantonio & O'Keefe, 2022), including acne severity (Bahadur *et al.* 2021). Intriguingly, in women with PCOS, an MI/DCI formulation with a higher DCI concentration (3.6:1 ratio) yielded superior reproductive outcomes – specifically, improved pregnancy rates and a reduced risk of ovarian hyperstimulation syndrome – compared with the standard 40:1 formulation (Mendoza *et al.* 2019). While the systemic effects of these insulin-sensitizing agents are well documented (Fedeli *et al.* 2023; Lentini *et al.* 2025), to our knowledge no prior study has investigated the impact of oral MI/DCI supplementation on the transcriptional regulation of insulin resistance pathway genes directly within acne-affected human skin. We therefore designed an exploratory, single-arm, uncontrolled, pre-post biopsy study to evaluate changes in *FOXO1*, *MTOR*, and *IGF1* mRNA expression in acne-involved skin of adults with PCOS or MetS after 12 weeks of oral MI/DCI supplementation in a 3.6:1 ratio.

METHODS

Study participants

This was an exploratory, single-arm, uncontrolled, pre-post biopsy study designed to generate hypothesis-forming data on cutaneous gene expression changes after MI/DCI supplementation. Forty-five adults aged ≥18 years with active inflammatory acne on their back were enrolled across three predefined subgroups of 15 subjects each – including (1) women with PCOS; (2) women with MetS without PCOS; and (3) men with MetS. PCOS was diagnosed according to the Rotterdam criteria (Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group, 2004), whereas MetS was defined by the harmonized criteria proposed by the International Diabetes Federation, the American Heart Association, and the National Heart, Lung, and Blood Institute (Alberti *et al.* 2009). Key exclusion criteria included (1) co-existing significant inflammatory or infectious skin diseases in the biopsy area; (2) use of systemic isotretinoin within the preceding 6–12 months; (3) use of systemic antibiotics, hormonal therapies for acne, or other systemic acne medications or food supplements within the preceding three months; (4) use of topical retinoids, antibiotics, or other topical acne treatments within the preceding 4–8 weeks; (5) known hypersensitivity to any component of the study supplement; (6) overt diabetes mellitus; (7) any serious or unstable systemic disease that could interfere

with study participation; and (8) pregnancy or lactation. The study protocol was approved by the local ethics committee (Studio Minoretti; approval number MA2511/04) and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants before enrollment.

Materials

The study nutraceutical (MYO-AC®; Bionativa SpA, Barberino Tavarnelle, Italy) consisted of tablets containing MI and DCI in a 3.6:1 ratio. Subjects were instructed to take one tablet in the morning and one in the evening for 12 consecutive weeks – orally, with water, at approximately the same times each day – providing a total daily dose of 1100 mg of MI and 300 mg of DCI.

Clinical and biochemical assessments

At baseline, body weight and height were measured with calibrated instruments, and body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Waist circumference was measured at the midpoint between the lower costal margin and the iliac crest at the end of normal expiration with a non-stretchable tape. Venous blood samples were collected in the morning after an overnight fast of at least 8 hours. Fasting plasma glucose was determined by the hexokinase enzymatic method, and fasting serum insulin was measured by chemiluminescence immunoassay. Insulin resistance was estimated by the homeostatic model assessment of insulin resistance (HOMA-IR), calculated as fasting insulin (μU/mL) × fasting glucose (mg/dL) / 405 (Sama *et al.* 2021). In the subgroup of men with metabolic syndrome, plasma total testosterone was quantified at baseline and at week 12 by chemiluminescent detection.

Endpoints

The prespecified primary endpoint was the within-subject change from baseline to week 12 in cutaneous mRNA expression of *FOXO1*, *MTOR*, and *IGF1* in acne-involved skin. Clinical response (Investigator Global Improvement rating) (Emanuele *et al.* 2012) and safety outcomes (adverse events and plasma testosterone in men) were defined as secondary endpoints.

Gene expression analyses

From each participant, two 4-mm punch biopsies of acne-involved skin on the back were collected – one before treatment initiation and one at the completion of the 12-week intervention. Biopsy specimens were immediately processed, and total RNA was extracted with the RNeasy Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Integrity of the isolated RNA was confirmed by agarose gel electrophoresis, and concentration was quantified by spectrophotometric analysis. Reverse transcription was performed on 1 μg of total RNA using the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA); cDNA samples were then stored at −20 °C until analysis. Gene expression was quantified by qRT-PCR on a Bio-Rad iQ5 Cycler (Bio-Rad). Reactions were carried out in a total volume of 25 μL containing 40 ng of cDNA, 400 nM each of forward and reverse primers, and iQ SYBR Green Supermix (Bio-Rad). The thermal cycling protocol comprised an initial denaturation step at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 10 s, annealing at 60 °C for 15 s, and extension at 72 °C for 30 s. The following primer pairs were used: *FOXO1* forward, 5'-GCCATGTAAGTCCCATCAGGA-3'; *FOXO1* reverse, 5'-ATCGGAACAAGAACGTGGAATC-3'; *MTOR* forward, 5'-GCATGAATCGGGATGATCG-3'; *MTOR* reverse, 5'-CTGCTGCTGTGTGATTTCTT-3'; *IGF1* forward, 5'-CTTCAGTTCGTGTGTGGAGACAG-3'; *IGF1* reverse, 5'-CGCCCTCCGACTGCTG-3'. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) served as the reference gene for normalization, with forward and reverse primers of 5'-GAAGGTGAAGGTCGGAGTC-3' and 5'-GAAGATGGTGATGGGATTTC-3', respectively. Fluorescence signals were processed with Bio-Rad iQ5 Optical System Software Version 2.0, and relative mRNA levels at each time point were expressed as $2^{-\Delta CT}$ values, where ΔCT represented the difference between the cycle threshold of the target gene and that of *GAPDH* (Emanuele *et al.* 2012). Pre-post changes were evaluated by comparing paired $2^{-\Delta CT}$ values statistically.

Statistical methods

Because of the exploratory design, no formal sample size calculation was performed; a total of 45 participants (15 per subgroup) was chosen pragmatically to ensure feasibility of paired biopsy collection and adequate precision around mean within-subject changes. Continuous variables are reported as mean ± standard deviation, whereas categorical variables are expressed as counts. Within-subject changes between baseline and week 12 were assessed by paired Student's t-test for normally distributed variables and by the Wilcoxon signed-rank test for variables that deviated from normality. Between-group comparisons of baseline characteristics across the three subgroups were conducted by one-way analysis of variance (ANOVA) for normally distributed variables and the Kruskal-Wallis test for skewed data. Clinical responses at week 12 were assessed by the Investigator Global Improvement rating on a five-point scale and reported as frequency distributions. Effect sizes for all paired comparisons were expressed as Cohen's *d* – calculated as the mean within-subject difference divided by the standard deviation of within-subject differences – with 95% confidence intervals (CIs) for the mean difference. Given the exploratory and hypothesis-generating nature of this study, no formal adjustment for multiple comparisons was applied across genes, time points, and subgroups. As a result, all subgroup-level and

Tab. 1. General characteristics of the study participants

Characteristic	Overall (n = 45)	Women with PCOS (n = 15)	Women with MetS (n = 15)	Men with MetS (n = 15)	*p*	Test
Age, years	36.7 ± 9.2	28.1 ± 5.0	39.3 ± 5.5	42.8 ± 8.2	<0.001	ANOVA
Body mass index, kg/m^2	29.9 ± 3.8	27.6 ± 2.7	31.4 ± 4.8	30.7 ± 3.2	0.015	ANOVA
Waist circumference, cm	95.5 ± 11.5	85.2 ± 5.7	99.0 ± 11.7	102.5 ± 10.8	<0.001	ANOVA
Fasting glucose, mg/dL	105.7 ± 16.3	94.6 ± 12.0	107.7 ± 13.2	114.9 ± 18.5	0.002	ANOVA
Fasting insulin, µIU/mL	17.9 ± 6.0	17.4 ± 4.8	18.5 ± 6.9	17.7 ± 6.3	0.87	Kruskal-Wallis
HOMA-IR	4.8 ± 2.0	4.1 ± 1.4	5.0 ± 2.2	5.1 ± 2.3	0.33	ANOVA

Data are presented as means ± standard deviations. Between-group comparisons by one-way ANOVA for normally distributed continuous variables and Kruskal-Wallis test for non-normally distributed variables. Abbreviations: HOMA-IR, homeostatic model assessment of insulin resistance; MetS, metabolic syndrome; PCOS, polycystic ovary syndrome; ANOVA, analysis of variance.

gene-specific analyses should be interpreted cautiously as descriptive signals rather than confirmatory inferential findings. All statistical analyses were carried out in SPSS 20.0 (IBM, Armonk, NY, USA), and two-sided p values < 0.05 were regarded as statistically significant.

RESULTS

Participants' characteristics

The general characteristics of the study participants are summarized in Table 1. All enrolled subjects (n = 45) completed the 12-week treatment protocol, with no premature discontinuations. The three subgroups differed significantly in age ($p < 0.001$), BMI ($p = 0.015$), and waist circumference ($p < 0.001$), with women with PCOS being younger and leaner than both MetS groups. Fasting glucose was also significantly lower in the PCOS subgroup compared to the metabolic syndrome subgroups ($p = 0.002$), whereas fasting insulin and homeostatic model assessment of insulin resistance (HOMA-IR) did not differ significantly among the three groups, indicating a comparable degree of insulin resistance across the study population.

Changes in cutaneous gene expression

After 12 weeks of oral supplementation with MI and DCI in a 3.6:1 ratio, significant changes were observed in the cutaneous expression of all three primary gene targets in paired skin biopsies obtained from acne-involved areas (Table 2). *FOXO1* mRNA expression in the overall cohort (n = 45) increased from 0.57 ± 0.19 at baseline to 0.70 ± 0.19 at week 12, corresponding to a mean change of +0.13 relative expression units (Cohen's $d = +1.13$; $p < 0.001$). The observed upregulation was consistent across all subgroups and ranged from +0.16 in women with PCOS ($p < 0.001$) and +0.16 in men with MetS ($p < 0.001$) to +0.07 in women with MetS without PCOS ($p < 0.05$). *MTOR* mRNA expression decreased from 3.43 ± 0.65 to 2.31 ± 0.55 in the overall cohort (mean reduction −1.12; Cohen's $d = -3.46$; $p < 0.001$). All three subgroups demonstrated significant reductions in *MTOR* expression (all $p < 0.001$). Cutaneous *IGF1* mRNA expression decreased from 2.77 ± 0.75 to 2.10 ± 0.66 in the overall cohort (mean change −0.67; Cohen's $d = -1.62$; $p < 0.001$). Reductions were observed across all metabolic phenotypes, with significant downregulation in women with PCOS ($p < 0.01$), women with MetS ($p < 0.001$), and men with MetS ($p < 0.001$).

Clinical response

At the end of the 12-week intervention, clinical response was evaluated using the Investigator Global Improvement scale, a five-point rating tool (Table 3). Most patients (82.2%) demonstrated clinical benefit, as indicated by a score of 1 or above. Specifically, 24 subjects (53.3%) were rated as "improved," 12 (26.7%) as "markedly improved," and one (2.2%) as "resolved." Eight subjects (17.8%) were rated as "unchanged," and none as "worsened." This pattern was consistent across all three metabolic subgroups.

Safety and tolerability

The study supplement was safe and generally well tolerated over the 12-week study period. No severe or serious adverse events occurred, and no participants discontinued the study due to adverse reactions. Two subjects (4.4%) reported mild, transient gastrointestinal discomfort (bloating and mild nausea) during the first two weeks of supplementation. These symptoms were self-limiting and did not require dose modification.

Plasma testosterone assessment in men with metabolic syndrome

In the subgroup of men with metabolic syndrome (n = 15), plasma testosterone concentration was quantified by chemiluminescence immunoassay to evaluate potential systemic endocrine effects of the nutraceutical. Mean baseline total testosterone was 5.27 ± 0.65 ng/mL. After 12 weeks of oral supplementation with MI and DCI in a 3.6:1 ratio, levels remained stable at 5.22 ± 0.63 ng/mL (mean change −0.05 ng/mL;

Tab. 2. Gene expression changes in acne areas from baseline to week 12

Group	n	mRNA	Baseline	Week 12	Δ	Cohen's *d*	95% CI for Δ	*p*	Test
Entire cohort	45	*FOXO1*	0.57 ± 0.19	0.70 ± 0.19	+0.13	+1.13	[+0.09, +0.16]	<0.001	Paired *t*-test
Women with PCOS	15	*FOXO1*	0.52 ± 0.11	0.68 ± 0.10	+0.16	+2.59	[+0.13, +0.20]	<0.001	Paired *t*-test
Women with MetS	15	*FOXO1*	0.62 ± 0.20	0.69 ± 0.19	+0.07	+0.63	[+0.01, +0.12]	<0.05	Paired *t*-test
Men with MetS	15	*FOXO1*	0.58 ± 0.23	0.74 ± 0.26	+0.16	+1.14	[+0.08, +0.24]	<0.001	Paired *t*-test
Entire cohort	45	*MTOR*	3.43 ± 0.65	2.31 ± 0.55	-1.12	-3.46	[-1.21, -1.02]	<0.001	Paired *t*-test
Women with PCOS	15	*MTOR*	3.48 ± 0.57	2.28 ± 0.41	-1.20	-3.95	[-1.37, -1.03]	<0.001	Paired *t*-test
Women with MetS	15	*MTOR*	3.48 ± 0.91	2.44 ± 0.73	-1.04	-3.50	[-1.19, -0.89]	<0.001	Wilcoxon
Men with MetS	15	*MTOR*	3.33 ± 0.42	2.22 ± 0.49	-1.11	-2.64	[-1.33, -0.90]	<0.001	Paired *t*-test
Entire cohort	45	*IGF1*	2.77 ± 0.75	2.10 ± 0.66	-0.67	-1.62	[-0.80, -0.55]	<0.001	Paired *t*-test
Women with PCOS	15	*IGF1*	2.70 ± 0.50	2.24 ± 0.69	-0.46	-0.87	[-0.74, -0.16]	<0.01	Paired *t*-test
Women with MetS	15	*IGF1*	2.87 ± 0.88	2.14 ± 0.68	-0.73	-2.65	[-0.87, -0.59]	<0.001	Wilcoxon
Men with MetS	15	*IGF1*	2.76 ± 0.84	1.92 ± 0.62	-0.84	-2.40	[-1.03, -0.64]	<0.001	Paired *t*-test

Data are presented as means ± standard deviations. mRNA expression levels were calculated by the $2^{-\Delta CT}$ method with *GAPDH* as the reference gene. Within-subject changes from baseline to week 12 were analyzed by paired Student's t-test or Wilcoxon signed-rank test. Cohen's *d* was calculated as the mean within-subject difference divided by the standard deviation of the within-subject differences. The 95% CI refers to the confidence interval for the mean difference (Δ). Abbreviations: MetS, metabolic syndrome; PCOS, polycystic ovary syndrome.

Cohen's $d = -0.12$; 95% CI, −0.28 to +0.17; $p = 0.638$, paired Student's *t*-test).

DISCUSSION

The main findings of this exploratory study can be summarized as follows. First, during 12 weeks of oral MI/DCI supplementation (3.6:1 ratio), we observed significant upregulation of *FOXO1* and significant downregulation of both *MTOR* and *IGF1* mRNA in acne-involved skin. Second, these changes were directionally consistent across all three metabolic subgroups. Third, clinical improvement was documented in 82.2% of participants. However, in the absence of a placebo or control group, we cannot distinguish the contribution of the intervention from the natural course of acne, regression to the mean, or non-specific factors such as expectation bias. Fourth, the intervention was safe and well tolerated, with only two subjects reporting mild transient gastrointestinal symptoms. Fifth, plasma testosterone concentrations in the male subgroup remained stable over the 12-week study period, indicating the absence of clinically relevant androgenic suppression.

The gene expression changes observed in the present investigation are consistent with a correction of the pathological signaling cascades that have been implicated in the metabolic pathogenesis of acne vulgaris.

Tab. 3. Change from baseline to week 12 in the Investigator Global Improvement Rating

Rating	Entire cohort (n = 45)	Women with PCOS (n = 15)	Women with MetS (n = 15)	Men with MetS (n = 15)
-1 (Worsened)	0 (0.0%)	0	0	0
0 (Unchanged)	8 (17.8%)	2	3	3
1 (Improved)	24 (53.3%)	8	7	9
2 (Markedly improved)	12 (26.7%)	4	5	3
3 (Resolved)	1 (2.2%)	1	0	0

Data are given as counts and overall percentages. Abbreviations: MetS, metabolic syndrome; PCOS, polycystic ovary syndrome.

In states of hyperinsulinemia, reduced nuclear *FOXO1* allows unopposed activation of *MTOR* and amplification of *IGF1*-mediated signaling, which in turn promote sebocyte proliferation, lipogenesis, and androgen receptor transactivation (Melnik & Zouboulis, 2013; Aktaş Karabay *et al.* 2020). The observed upregulation of *FOXO1* and concomitant reduction in *MTOR* and *IGF1* expression during MI/DCI supplementation are consistent with a partial normalization of insulin resistance–related signaling in acne-involved skin. While causality cannot be established from this uncontrolled design, these associative changes are in accordance with current models in which improved insulin signaling attenuates *MTOR*-driven sebocyte activation (Monfrecola *et al.* 2016) and *IGF1*-mediated pathways (Gu *et al.* 2022). The magnitude of the *MTOR* reduction was particularly large across the entire cohort (Cohen's $d = -3.46$) and was uniformly observed in all three subgroups. This finding is notable because *MTOR* occupies a central position in the integration of nutrient and hormonal signals within the sebaceous gland, and its pharmacological inhibition has been proposed as a therapeutic target in acne (Monfrecola *et al.* 2016; Kitano *et al.* 2024). The present data therefore suggest that oral inositol supplementation may represent a non-pharmacological approach potentially capable of attenuating *MTOR*-related signaling in acne-involved skin of patients with concurrent metabolic conditions. However, the *FOXO1* response, while statistically significant in all subgroups, showed greater heterogeneity in effect size across metabolic phenotypes. The largest effect was observed in women with PCOS (Cohen's $d = +2.59$), followed by men with MetS (Cohen's $d = +1.14$), whereas the effect in women with MetS without PCOS was more modest (Cohen's $d = +0.63$). These differences may reflect variability in the local hormonal milieu and tissue-level insulin sensitivity across metabolic phenotypes. In PCOS, the combined burden of hyperandrogenism and hyperinsulinemia may render cutaneous *FOXO1* expression particularly susceptible to correction by insulin-sensitizing agents (Xu & Wang, 2021), whereas in women with MetS alone – who are typically older and exhibit a different endocrine profile (Krentowska & Kowalska, 2022) – the response may be attenuated by additional age-related and inflammatory factors.

The choice of a 3.6:1 MI-to-DCI ratio in this study warrants comment. The physiological plasma ratio of the two stereoisomers has been estimated at approximately 40:1, and most clinical studies in PCOS have adopted this proportion (Colak *et al.* 2020; Pustotina *et al.* 2024). However, evidence from reproductive endocrinology suggests that formulations with a relatively higher DCI content may be advantageous in certain clinical settings characterized by pronounced insulin resistance (Mendoza *et al.* 2019). In insulin-resistant tissues, the epimerase-mediated conversion of MI to DCI is impaired, resulting in a relative DCI deficit (Gambioli *et al.* 2021). In this scenario, a formulation that supplies a greater proportion of DCI may compensate more effectively for this enzymatic deficiency – including within the pilosebaceous unit – though this hypothesis remains speculative and warrants evaluation in dose-finding studies that compare different MI/DCI ratios in acne-prone populations. The comparable degree of insulin resistance at baseline across the three subgroups – as indicated by non-significantly different fasting insulin and HOMA-IR values – provided a relatively uniform metabolic substrate for the assessment of the nutraceutical. Although the subgroups differed significantly in age, BMI, and waist circumference, these differences did not prevent consistent directionality in gene expression changes. It is therefore possible that the degree of peripheral insulin resistance, rather than the specific metabolic diagnosis, contributes to cutaneous pathway modulation by inositols – a hypothesis that warrants evaluation in larger studies. Future investigations with larger sample sizes should investigate whether baseline HOMA-IR can serve as a predictor of gene expression response.

From a clinical standpoint, the response data indicate that most participants experienced measurable improvements over 12 weeks, with similar patterns across all three metabolic subgroups. These parallel trends between clinical ratings and molecular changes are hypothesis-generating and suggest that MI/DCI merits further evaluation as a potential adjunctive option in metabolically compromised patients with acne, ideally within randomized, controlled trial frameworks. Notably, the positive clinical trend was consistently observed across all three metabolic subgroups – a finding that lends external validity to the skin molecular findings. The absence of any subject rated as worsened further supports the safety profile of the intervention in this population. In addition, the stability of plasma testosterone concentrations in men with MetS over the study period (Cohen's $d = -0.12$; $p = 0.638$) has clinical relevance. While androgen suppression would represent an unacceptable side effect in male patients, our data provide preliminary reassurance that MI/DCI supplementation at the doses employed in this study is not associated with a detectable change in circulating testosterone in this male MetS cohort. The mechanism by which the nutraceutical modulated cutaneous gene expression without altering circulating testosterone in male participants is likely related to the local – rather than systemic – nature of inositol-mediated insulin sensitization within the male skin.

We acknowledge several limitations to our investigation. First, the study followed a single-arm, open-label, pre-post design without a placebo control group. The absence of a control arm implies that spontaneous fluctuation, regression to the mean, and expectation bias cannot be excluded as potential contributors to both gene expression and clinical changes; therefore, the findings should be considered exploratory. Second,

the sample size of 45 subjects (15 per subgroup) was selected on an exploratory basis without a formal power calculation. While the magnitude of the observed effect sizes – particularly for *MTOR* – was large, these findings require confirmation in adequately powered, randomized, placebo-controlled studies. Third, gene expression was measured in biopsies from acne-involved skin on the back, and caution should be exercised when extrapolating these results to other anatomical sites such as the face. Fourth, the study evaluated gene expression at the mRNA level only; protein-level assessments and functional assays would provide additional mechanistic depth. Fifth, the 12-week observation period may have been insufficient to capture the full trajectory of clinical response, and longer follow-up studies with serial biopsy time points would be informative. Sixth, no correction for multiple comparisons was applied to the subgroup-level gene expression analyses, and the possibility of inflated type I error rates should be considered when interpreting borderline significant results. Finally, all participants had PCOS or MetS with evidence of insulin resistance, and biopsy sampling was restricted to acne on the back. As such, these findings may not extrapolate to lean adolescents with facial-predominant acne, to patients without metabolic abnormalities, or to other anatomical sites.

In conclusion, oral supplementation with MI and DCI in a 3.6:1 ratio for 12 weeks was associated with significant modulation of the insulin-resistance signaling axis in acne-involved skin of adult subjects with PCOS or metabolic syndrome. *FOXO1* expression increased while *MTOR* and *IGF1* expression decreased across all metabolic phenotypes, and these molecular changes were accompanied by clinical improvements in most participants. The treatment was safe, well tolerated, and did not alter plasma testosterone in men. Collectively, these preliminary data provide a molecular rationale for further evaluation of combined MI/DCI supplementation as an adjunctive approach in acne associated with metabolic comorbidities. Future studies should include randomized, placebo-controlled designs, direct comparison of different MI/DCI ratios, longer follow-up, and incorporation of protein-level and functional readouts to clarify the clinical and mechanistic relevance of the observed gene expression changes.

CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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