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ORIGINAL RESEARCH

Prospective Comparative Study of an Oral Synbiotic and a Myoinositol-Based Herbal Supplement in Modifying Hormone Levels and the Gut Microbiome in Non-cystic Acne

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ABSTRACT

***Introduction*:** Acne pathogenesis is multifactorial, involving systemic factors including gut dysbiosis, hormones, and chronic inflammation. Probiotics, myoinositol, and plant-derived molecules may modulate acne by targeting these factors. The objective is to compare a synbiotic containing herbs against a myoinositol-based herbal supplement on how they influence acne, the gut microbiome, short chain fatty acids (SCFAs), and hormonal profiles.

***Methods*:** This was an 8-week, randomized study involving 36 male and female patients aged 12 to 45 years with non-cystic acne. Subjects received either a synbiotic or a myoinositol-based herbal supplement (MBHS). Acne lesions were counted, stool samples were collected for gut microbiome and SCFA analyses, and hormone collections were performed at baseline, 4, and 8 weeks.

***Results*:** Several gut bacteria increased by at least threefold at both week 4 and 8 in the synbiotic (*Erysipelatoclostridium merdavium*, *Blautia argi*, *Faecalibacterium prausnitzii*, *Prevotella copri*, *Streptococcus sp001556435*, *Blautia sp900541955*) and MBHS group (*Megamonas funiformis*, *Ligilactobacillus ruminis*, *Prevotella ssp015074785*, *Prevotella copri*, *Gca-900199835 sp900176495*). Acne lesion counts decreased significantly in both groups at week 4 ($p<0.0001$) and week 8 (synbiotic, $p<0.0001$; MBHS, $p<0.0001$). There were significant and trending increases in stool and plasma SCFAs in both cohorts at week 4 and 8. After 8 weeks of MBHS, 17-OHP and androstenedione significantly decreased from 27.3 to 11.3 pg/ml ($p=0.001$) and 94.9 to 68.0 pg/ml ($p=0.04$), respectively.

***Conclusion*:** Both the synbiotic and MBHS improved gut health, augmented SCFAs, and

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reduced lesion counts in those with non-cystic acne. The MBHS may act by reducing hormone levels of 17-OHP and androstenedione.
Clinical Trial Registration: www.clinicaltrials.gov (NCT05919810).

Keywords: Noncystic acne; Probiotic; Myoinositol; Guggul; EGCG; Silymarin; Herbal supplement; Short chain fatty acids; Gut microbiome

Key Summary Points

In this randomized clinical trial of 36 patients with non-cystic acne, both the synbiotic and the myoinositol-based herbal supplement significantly improved gut health, hormones, and acne lesion counts.

Several beneficial gut bacteria increased, pathogens decreased, and circulating and stool short chain fatty acids were augmented in both cohorts.

After 8 weeks of supplementation with the myoinositol-based herbal supplement, 17-OHP and androstenedione were significantly decreased, suggesting that myoinositol may regulate hormone levels of testosterone precursors.

INTRODUCTION

Acne is a chronic inflammatory disease of the pilosebaceous unit characterized by comedones, papules, pustules, nodules, scarring, and post-inflammatory hyperpigmentation primarily affecting the face, upper trunk, and extremities [1]. The prevalence of acne is greater than 85% among adolescents and young adults and is associated with a significant psychosocial burden [2].

Acne pathogenesis is multifactorial, with traditionally described mechanisms focusing on local factors including sebum overproduction, infiltration of inflammatory cells, hyperplasia of sebaceous glands due to androgenic influence, hyperkeratinization and ductal obstruction of follicles, and overgrowth of *Cutibacterium acnes* [3]. However, emerging evidence demonstrates the involvement of systemic and distant factors on acne pathogenesis and severity. For example, shifts in the gut microbiome [4], circulating short chain fatty acids (SCFAs) [5], lipid composition [6], circulating hormones and metabolites [7], and stress [8, 9] have all been shown to influence acne pathogenesis.

Conventional treatment modalities for acne target local factors, aiming to reduce microbial burden, seborrhea, prevent hyperkeratinization, and downregulate androgenic effects. Therapeutic options often include topical and oral antibiotics (clindamycin, erythromycin, tetracyclines), retinoids (adapalene, tretinoin, tazarotene, isotretinoin), among other antimicrobial and keratolytic options (benzoyl peroxide, azelaic acid, sulfacetamide sodium-sulfur) [1]. Since acne has a hormonal influence, medications such as spironolactone and oral contraceptives are often utilized. Although efficacious, many of these options carry side effects and risks. For example, the use of oral antibiotics increases the risk for bacterial resistance, gastrointestinal symptoms, yeast infections, and overgrowth of pathogenic bacteria [1]. Oral spironolactone cannot be used in male patients because of risk for gynecomastia [10]. Given these concerns, there has been growing interest in the role of alternative therapies for acne, many of which target systemic or distant factors and may act synergistically with pharmaceutical options [11]. Recent developments in acne treatment regimens expand on the currently available regimens to include dietary changes, probiotics, nutraceuticals, and lifestyle modifications [1].

Numerous studies have demonstrated the bidirectional relationship between the gut microbiome and skin health, termed the gut–skin axis [12]. Intestinal microorganisms modulate the function and composition of the innate and adaptive immune systems, thereby maintaining skin homeostasis and modulating inflammatory skin conditions such as atopic dermatitis, psoriasis, acne, and hidradenitis suppurativa [13]. During periods of stress, the gut microbiome has also been shown to produce neurotransmitters with inflammatory effects on

the body [14]. Moreover, intestinal microbiota modify the production of secondary metabolites, like SCFAs, further triggering downstream pathways that influence overall homeostasis and skin health [5]. Consequently, dysbiosis of the gut microbiome has been associated with worsening of inflammatory skin conditions, particularly acne [15, 16].

The use of oral probiotics has been thought to have therapeutic potential in skin diseases by ameliorating gut dysbiosis. Notably, several in vitro studies have demonstrated efficacy of some strains in inhibiting acne pathogenesis. For example, strains of *Lactobacillus* and *Bifidobacterium* were shown to adhere to keratin and inhibit biofilm formation of pathogenic bacteria [17], as well as produce bacteriocins that inhibit the growth of *C. acnes* [14, 18]. In humans, clinical trials suggest that oral probiotic supplementation normalizes cutaneous expression of insulin-like growth factor 1 (IGF-1) and forkhead box protein O1 (Fox01) genes [19], increases levels of anti-inflammatory interleukin (IL)-10 [20], decreases the number of acne lesions, rate of desquamation, sebum secretion, and presence of *C. acnes* [21]. Interestingly, one study found that combining an oral probiotic (*Bifidobacterium breve* BR03 DSM 16604, *Lacticaseibacillus casei* LC03 DSM 27537, and *Ligilactobacillus salivarius* LS03 DSM 22776) with a botanical extract (containing lupeol from *Solanum melongena* L. and *Echinacea* extract) was more efficacious in reducing total facial lesion count compared to placebo, oral probiotic, and botanical extract alone in individuals with mild to moderate acne over 8 weeks [21].

Apart from probiotics, several other ingredients have also demonstrated promise for support in acne and in management of gut health and stress. *Silybum marianum* fruit extract (SMFE), otherwise known as milk thistle, interferes with comedone formation by modulating the expression of keratins 75 and 79 and proteins produced by sebaceous stem cell lineage [22]. Myoinositol plays a significant role in insulin signaling and hormonal synthesis, thereby exerting effects on hormonal regulation, metabolic health, and glucose homeostasis [23, 24]. Berberine has been shown to improve metabolic health and acne in those with polycystic ovarian syndrome [25]. *Ocimum sanctum* L. (holy basil) has been shown to have antimicrobial activity against *C. acnes* in an in vitro study [26]. L-Theanine supplementation has been shown to reduce stress-related symptoms in healthy adults [27]. Moreover, plant-derived compounds may possess prebiotic effects and influence the gut microbiome and modulate acne severity via mechanisms related to the intestinal microbiota [28].

Previous studies have not yet investigated whether a synbiotic can address the gut–skin axis, inflammation, and hormonal pathways related to acne pathogenesis. Here, we explore the efficacy and proposed mechanisms of an oral synbiotic and a myoinositol-based herbal supplement in a head-to-head clinical study to assess their influence on mild-to-moderate non-cystic acne, gut health, SCFAs, and circulating hormones.

METHODS

Investigational Product and Application

The synbiotic supplement used in this study is commercially available from Codex Labs (San Jose, CA, USA) under the trade name Shaant Clear Skin and contains the following ingredients: *Bifidobacterium lactis*, *Lactobacillus acidophilus*, *Bacillus coagulans*, guggul extract (*Commiphora mukul*), epigallocatechin gallate (EGCG), hypromellose (capsule), rice extract blend, gum fiber blend, organic rice fiber, fiber blend (rice fiber, oat fiber, sunflower lecithin), and silicon dioxide. Subjects who were allocated to this group were instructed to take two capsules (77 mg) daily with water.

The myoinositol-based herbal supplement used in this study is commercially available from Codex Labs (San Jose, CA, USA) under the trade name Shaant Skin De-Stress and contains the following ingredients: myoinositol, folate, pantothenic acid, sodium, methylsulfonylmethane, organic holy basil extract (*Ocimum sanctum* leaves), turmeric root extract (*Curcuma longa*), (50 mg curcuminoids), barberry root extract (*Berberis vulgaris*), L-theanine, milk thistle extract (*Silybum marianmum*, 140 mg Silymarin), natural

flavors, citric acid, organic rice fiber, sea salt, stevia leaf extract. Subjects who were allocated to this group were instructed to mix one scoop (5.6 g) into a single serving of water (8 oz. or 240 mL) once daily.

Inclusion and Exclusion Criteria

Inclusion criteria: male and female patients between the ages of 12 and 45 years. The presence of non-cystic, mild to moderate acne based on investigator global assessment (grade 2 or 3) and presence of at least 10 inflammatory lesions and at least 5 non-inflammatory lesions.

Exclusion criteria: the presence of severe acne as noted by the investigator global assessment (grade 4) or the presence of cystic acne. Those who are unwilling to discontinue oral probiotic-based supplementation, or supplement containing ingredients found in the study's oral product 1 month prior to enrollment. Those who are unwilling to discontinue topical antibiotics and topical benzoyl peroxide for 2 weeks prior to enrollment. Those who are unwilling to keep their non-prescription facial regimen the same throughout the study. Individuals who have been on an oral antibiotic for acne within a month prior to enrollment. Individuals who are pregnant or breastfeeding. Individuals who have changed any of their hormonal based contraception or therapies within 3 months prior to enrollment. Individuals with use of isotretinoin within 3 months prior to enrollment. Individuals on finasteride or dutasteride. Current tobacco smoker or a tobacco smoking history.

Study Design and Recruitment

This study was an 8-week, randomized, head-to-head clinical trial conducted between July 2023 and July 2024. The study was conducted according to the guidelines of the Declaration of Helsinki, approved by the Allendale Institutional Review Board (CB_Acne_Supp, June 26, 2023), and registered on www.clinicaltrials.gov (NCT05919810). All participants or guardians (in the case of minor) provided signed informed consent prior to participation and all minors provided signed informed assent. All participants or guardians (in the case of minor) provided written consent for their photographs to be used in publication. Male and female patients, ages 12 to 45 years, in the greater Sacramento area were recruited and screened for eligibility. All study procedures were completed at Integrative Skin Science and Research in Sacramento, CA. A priori randomization was performed with a computer-based randomization program and eligible subjects were assigned to groups through the use of blinded sealed envelopes by the study coordinator. Study visits occurred at baseline, week 4, and week 8.

Stool Sample Collection, DNA Extraction, and 16S rRNA Sequencing

All subjects were provided with pre-assembled stool sample collection kits and were instructed to bring back samples collected within 72 h of their baseline, week 4, and week 8 visits.

Full Length 16S rRNA Sequencing

Total DNA extraction was performed using the MagMax Microbiome Ultra Nucleic Acid Isolation Kit (Applied Biosystems) and the KingFisher Flex Purification System (Thermo Fisher Scientific) according to the manufacturer's instruction. Cells were processed using the homogenizer MP FastPre-24 5G (MP Biomedical). The total DNA recovered was quantified using the Qubit 4 Fluorometer (Thermo Fisher Scientific) and the dsDNA high-sensitivity (HS) kit.

The full-length 16S rRNA gene (approximately 1500 bp in length) was amplified with some modifications to the previously described method [29]. The polymerase chain reaction (PCR) was carried out in 25 µl total volume containing 12.5 µl LongAmp Taq 2× Master Mix (New England Biolabs), 400 nM primers concentration, and 8.5 µl template DNA. Primers 27f (5′-TTTCTGTTGGTGCTGATATTGC-AGRGTTYGATYMTGGCTCAG-3′) and 1492r (5′-ACTTGCCTGTCGCTCTATCTTC-CGGTTACCTTGTTACGACTT-3′) were used to amplify the full-length 16S gene. The PCR amplification program was set as follows: initial denaturation at 95 °C for 4 min, followed by 30 cycles at 95 °C for 20 s,

51 °C for 30 s, and 65 °C for 4 min and a final extension 65 °C (5 min). Successfully amplified samples were cleaned with 0.6× Agencourt AMPure XP beads (Beckman Coulter) and resuspended in 15 µl of nuclease-free water.

Each sample was assigned a unique molecular barcode and added in a second PCR reaction using a modified version of the "PCR Barcoding Expansion 1–96 kit" (Oxford Nanopore Technologies). This barcoding PCR was carried out with 12.5 µl LongAmp Taq 2× Master Mix (New England Biolabs), 0.5 µl barcoding primer mix (10 µM), and 70 fmol 16S amplicons from the previous reaction. The PCR thermal profile included an initial denaturation at 95 °C for 3 min; 12 cycles of 95 °C for 15 s, 62 °C for 15 s, and 65 °C for 4 min; and a final extension at 65 °C for 15 min. The barcoded samples were pooled, and sequencing adapters were added according to ligation sequencing kit SQK-LSK114 instructions (Oxford Nanopore Technologies). Briefly, 12.5 µl Ligation Buffer, 5 µl Quick T4 Ligase, and 2.5 µl Ligation Adapter were added to the pool, and the reaction was incubated at room temperature for 10 min. Lastly, 10 fmol of DNA library was gently loaded onto an R10.4.1 flow cell (Oxford Nanopore Technologies). Sequencing was performed using a GridION Mk1b device (Oxford Nanopore Technologies) with live basecalling in super accurate mode (quality threshold Q12).

Data Analysis Workflow

FastQ reads were pre-processed with cutadapt v. 3.5 to remove sequencing adapters and filtered with the dada2 v. 1.28.0 R package [30]. Reads ranging from 1200 and 1800 nucleotides in length were retained. Chimeras were detected and filtered out using Minimap2 v. 2.16 and yacrd v. 1.0.0 tools. Microbial taxonomy was determined using emu v. 3.4.4 [31] mapping filtered fastQ reads against the reference Genome Taxonomy Database (GTDB) database [32].

SCFA Measurement: Stool and Plasma

All subjects were provided with pre-assembled stool sample collection kits and were instructed to bring back samples collected within 72 h of their baseline, week 4, and week 8 visits. Subjects underwent venipuncture at baseline, week 4, and week 8. The collection tubes were centrifuged, and the supernatant plasma was selectively collected and stored at – 80 °C until they were shipped for analysis.

SCFAs in stool and plasma were quantified by Metabolon (Morrisville, NC, USA). Stool or plasma samples were analyzed for the following SCFAs: acetic acid (C2), propionic acid (C3), isobutyric acid (C4), butyric acid (C4), 2-methylbutyric acid (C5), isovaleric acid (C5), valeric acid (C5), and caproic acid (hexanoic acid, C6). SCFAs were analyzed by the Metabolon Method TAM148. Prior to analysis, individual analytes were preset to target standard concentrations and calibrated. Then, all samples were spiked with stable labelled internal standards and subsequently induced to protein precipitation with an organic solvent. The sample was centrifuged, and an aliquot of the supernatant was derivatized and injected onto an Agilent 1290/SCIEX QTrap 5500 LC MS/MS system (with a C18 reverse-phase UHPLC column). The settings for the mass spectrometer were set to negative mode with electrospray ionization. Briefly, the peak area of each individual analyte product's ions is measured and compared against the peak area of corresponding internal standards. SCFAs were then quantified by performing a weighted linear squares regression analysis. LC–MS/MS raw data was collected using SCIEX software Analyst 1.7.3 and processed with SCIEX OS-MQ software v3.1.6. Microsoft Excel for Microsoft 365 MSO was utilized to perform data reduction.

Hormone Quantification

This study quantified hormones from saliva rather than serum because previous studies that compared salivary to serum-based collections of hormones determined that both collection methodologies were similar [33]. Subjects were given saliva collection kits (ZRT Laboratory, Beaverton, OR, USA) at screening, baseline, and week 4 to bring back at baseline, week 4, and week 8, respectively. Each kit contained specific instructions on how to collect four samples of saliva, label tubes, and complete paperwork.

The first sample was collected upon waking up for the day, the second sample before lunch, third before dinner, and fourth right before bedtime. Subjects were instructed to complete their collections within 24 h prior to their next visit. Upon retrieval, samples were kept frozen at −80 °C prior to shipping and quantification by ZRT Laboratory (Beaverton, OR, USA). Salivary hormones analyzed included estradiol, estrone, DHEA, cortisol, cortisone, progesterone, 17-hydroxyprogesterone (17-OHP), androstenedione, aldosterone, and testosterone.

Investigator's Global Assessment and Lesion Counting

A board-certified dermatologist performed acne lesion counts for inflammatory and non-inflammatory lesions at screening, baseline, week 4, and week 8. The Investigator's Global Assessment (IGA) was utilized to calculate and grade acne severity at these same visits.

Facial Photography

Facial photographs were obtained by using high-resolution digital photographic tools such as the BTBP 3D imaging Photography System (BrighTex Bio-Photonics, LLC, San Jose, CA, USA) at screening, baseline, week 4, and week 8.

Statistical Analysis

Parametric statistical analyses were performed using Student's *t* test to assess within-group (two-tailed, paired) and between-group (two-tailed, unpaired) differences. Any values of $p<0.05$ were considered statistically significant and $p<0.10$ was considered a trend.

Statistical analysis of microbiome abundance was performed using RStudio v. 524 (R v. 4.3.2). Community analysis was performed using phyloseq R package v. 1.46 evaluating alpha diversity indexes. The Wilcoxon rank sum test was implemented to evaluate significative differences. Beta diversity was evaluated by running a principal coordinate analysis (PCoA) based on Bray–Curtis dissimilarity index. Significant differences in beta diversity were tested using permutational multivariate analysis of variance (PERMANOVA) test (adonis2 function, vegan package).

RESULTS

Subject Demographics

Thirty-six participants enrolled into this study with 18 subjects in each group (oral synbiotic and myoinositol-based herbal supplement). Among all 36 participants, 83.3% ($n=30$) were female and 16.7% were male ($n=6$). Within the oral synbiotic group, 88.9% ($n=16$) were female and 11.1% ($n=2$) were male. Within the myoinositol-based herbal supplement group, 77.8% ($n=14$) were female and 22.2% ($n=4$) were male. The average age among all participants was 22.7 ± 7.8, with an average of 22.9 ± 8.8 in the oral synbiotic group and an average of 22.5 ± 6.9 in the myoinositol-based herbal supplement group. The average baseline IGA in the oral synbiotic group was 2.9 ± 0.3 and the average baseline IGA in the myoinositol-based herbal supplement group was 2.7 ± 0.5. Two participants dropped out of the oral synbiotic group and four participants dropped out of the myoinositol-based herbal supplement group after baseline. A total of 30 participants completed the entire study, with 16 and 14 subjects in the oral synbiotic and myoinositol-based herbal supplement groups, respectively. Figure 1 demonstrates the flow of participants throughout the study.

Changes in Gut Microbiome

Shannon Diversity

The overall Shannon diversity in both the synbiotic and the myoinositol-based herbal supplement groups was unchanged at baseline, week 4, and week 8 (Fig. 2), demonstrating that neither intervention had a substantial effect on the gut microbial community.

Shifts in Relative Abundance

The relative abundance of several bacteria increased by at least threefold at both week 4 and week 8 in the synbiotic group including *Erysipelatoclostridium merdavium*, *Blautia argi*,

Faecalibacterium prausnitzii, *Prevotella copri*, *Streptococcus sp001556435*, and *Blautia sp900541955* (Fig. 3a). Bacteria that increased by at least threefold in the synbiotic group at week 8 are shown in Fig. 3b and the percent decreases in relative abundance of pathogenic species (*Clostridioides difficile*, *Collinsella sp002232035*, *Collinsella sp900542275*, *Collinsella tanakaei*, and *Bilophila wadsworthia*) are shown in Fig. 3c. There were increases in several beneficial species, continued microbial shifts by week 8, and a reduction in potentially harmful bacteria, suggesting a positive shift in the gut microbiome.

The relative abundance of several bacteria increased by at least threefold at both week 4 and week 8 in the myoinositol-based herbal supplement group including *Megamonas funiformis*, *Ligilactobacillus ruminis*, *Prevotella ssp015074785*, *Prevotella copri*, and *Gca-900199835 sp900176495* (Fig. 4a). Bacteria that increased by at least threefold in the myoinositol-based herbal supplement group at week 8 only are shown in Fig. 4b and

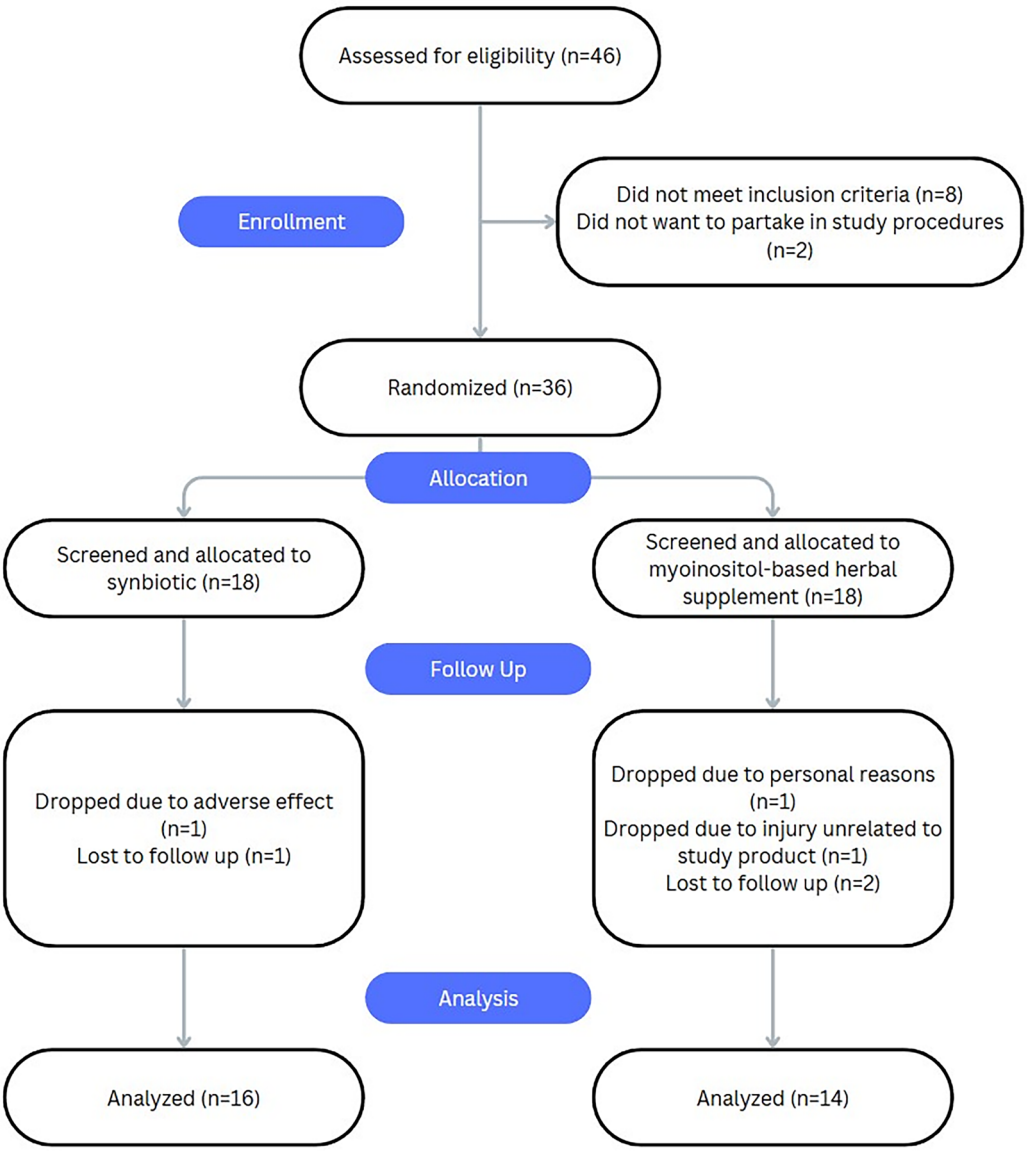

Fig. 1 Consolidated standards of reporting trials (CONSORT) diagram

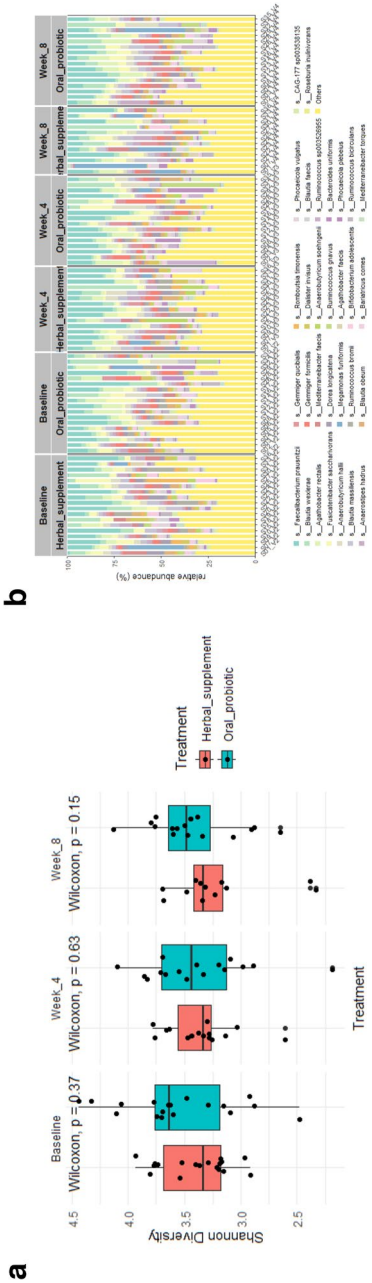

Fig. 2 **a** The Shannon diversity was not significantly different between or within the synbiotic or the myoinositol-based herbal supplement groups. **b** The top 30 species taxa are depicted for baseline, week 4, and week 8 for the synbiotic and the myoinositol-based herbal supplement groups

the percent decreases in relative abundance of pathogenic species (*Clostridioides difficile*, *Collinsella sp002232035*, *Collinsella sp900542275*, *Collinsella tanakaei*, and *Bilophila wadsworthia*) are shown in Fig. 4c. Similar to the synbiotic, these findings suggest that the myoinositol-based herbal supplement may promote a healthier gut microbiome by enhancing beneficial bacteria and reducing harmful species.

Lesion Counts

Non-inflammatory lesion counts decreased 29.1% ($p<0.0001$) and 28.6% ($p<0.0001$) in the synbiotic and myoinositol-based herbal supplement groups at week 4, respectively. At week 8, non-inflammatory lesion counts decreased 49.2% ($p<0.0001$) and 62.1% ($p<0.0001$) in the synbiotic and myoinositol-based herbal supplement groups, respectively. Differences between the two cohorts at week 4 and week 8 were not significant. These results are shown in Fig. 5a.

Inflammatory lesion counts decreased 44.6% ($p<0.0001$) and 55.2% ($p<0.0001$) in the synbiotic and myoinositol-based herbal supplement groups at week 4, respectively. At week 8, inflammatory lesion counts decreased 58.2% ($p<0.0001$) and 61.1% ($p<0.001$) in the synbiotic and myoinositol-based herbal supplement groups, respectively. Differences between the two cohorts at week 4 and week 8 were not significant. These results are shown in Fig. 5b.

While both interventions led to significant reductions in both non-inflammatory and inflammatory lesion counts, the myoinositol-based herbal supplement group showed a slightly greater reduction in lesion counts at week 8 compared to the synbiotic group. Between-group analysis demonstrated that the differences between the two groups were not statistically significant.

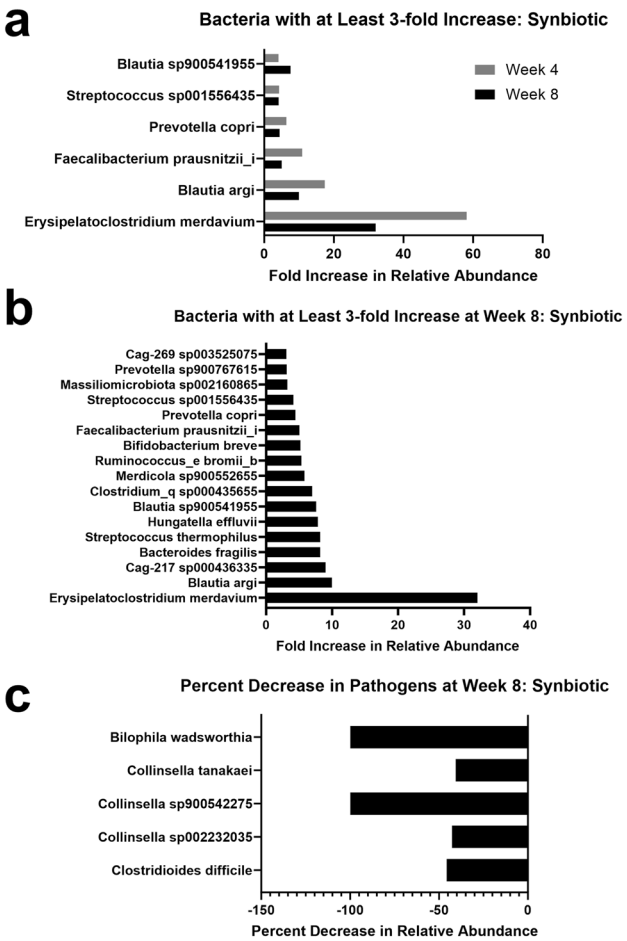

Fig. 3 In the synbiotic group, **a** bacteria with at least threefold increase at both week 4 and 8, **b** bacteria with at least threefold increase at week 8 only, and **c** percent decrease in pathogenic bacteria at week 8

Stool and Plasma SCFAs

Synbiotic Supplement

After 4 weeks of synbiotic supplementation, stool concentrations of isovaleric acid trended up by 47% ($p = 0.09$). After 8 weeks of synbiotic supplementation, stool concentrations of acetic acid, butyric acid, and propionic acid all significantly increased by 20% ($p = 0.02$), 51% ($p = 0.013$), and 31% ($p = 0.02$), respectively. These results are shown in Fig. 6a.

Plasma concentrations of hexanoic acid significantly increased by 39% ($p = 0.04$) after 4 weeks of supplementation. After 8 weeks of supplementation, plasma concentrations of propionic acid significantly decreased by 26% ($p = 0.03$). These results are shown in Fig. 6b.

Stool SCFAs increased over time, with statistically significant rises by week 8 for acetic, butyric, and propionic acids. Plasma SCFAs showed mixed trends, with hexanoic acid increasing at week 4 but propionic acid decreasing at week 8. The increase in stool SCFAs may suggest enhanced microbial fermentation

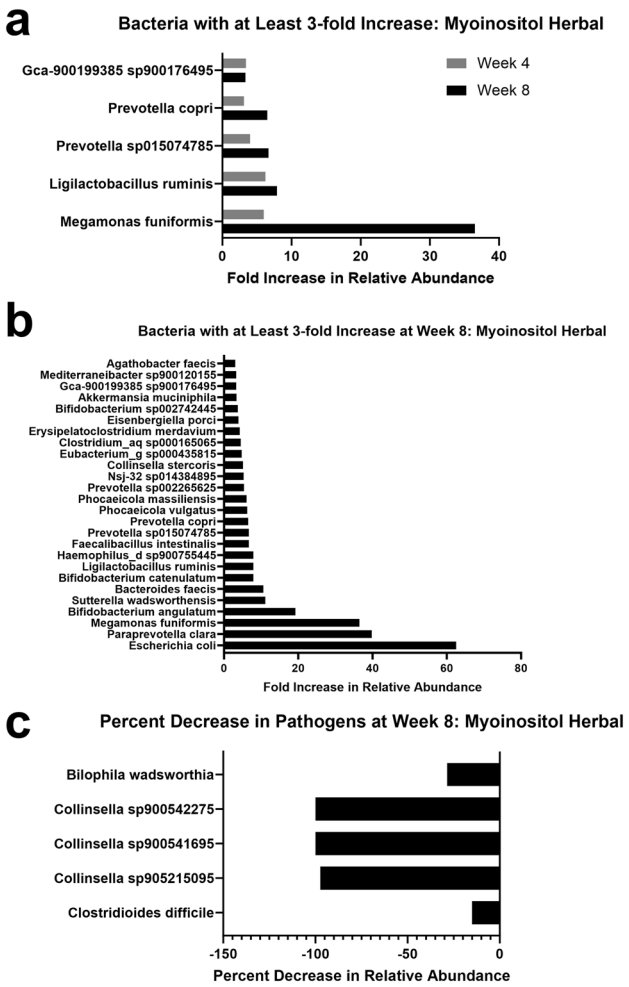

Fig. 4 In the myoinositol-based herbal supplement group, **a** bacteria with at least threefold increase at both week 4 and 8, **b** bacteria with at least threefold increase at week 8 only, and **c** percent decrease in pathogenic bacteria at week 8

activity following synbiotic supplementation. Overall, the data indicate that synbiotic supplementation significantly modulates SCFA profiles in both stool and plasma over time.

Myoinositol-Based Herbal Supplement

After 4 weeks of supplementation with the myoinositol-based herbal supplement, stool concentrations of acetic acid and valeric acid significantly increased by 99.9% ($p = 0.04$) and 79.7% ($p = 0.04$), respectively. Moreover, 2-methylbutyric acid, butyric acid, hexanoic acid, isobutyric acid, and propionic acid trended up by 144.5% ($p = 0.05$), 185.8% ($p = 0.05$), 897.5% ($p = 0.08$), 109.9% ($p = 0.09$), and 87.6% ($p = 0.09$), respectively. After 8 weeks of myoinositol-based herbal supplementation, stool concentrations of acetic acid significantly increased by 110.7% ($p = 0.03$). Butyric acid, hexanoic acid, and propionic acid trended up by 183.9% ($p = 0.05$), 609.8% ($p = 0.09$), and 128.6% ($p = 0.08$), respectively. These results are shown in Fig. 7a.

Plasma concentrations of acetic acid significantly increased by 97.6% ($p = 0.04$) and isovaleric acid trended up by 81.8% ($p = 0.05$) after 4 weeks of supplementation. After 8 weeks of supplementation, plasma concentrations of acetic acid trended up by 28.5% ($p = 0.08$) and propionic acid trended down by 22.5% ($p = 0.08$). These results are shown in Fig. 7b.

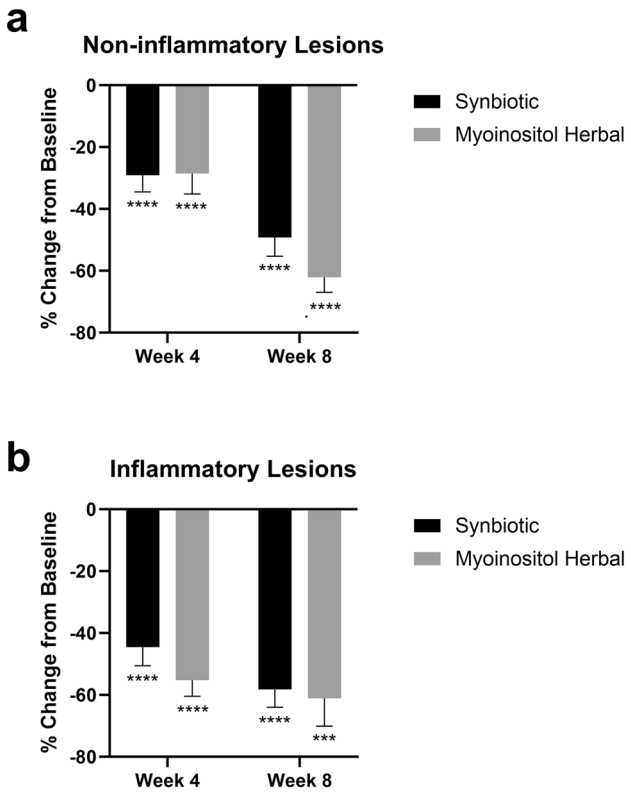

Fig. 5 Percent change in **a** non-inflammatory and **b** inflammatory lesion counts in the synbiotic and myoinositol-based herbal supplement groups at week 4 and week 8 compared to baseline. ***$p < 0.001$, ****$p < 0.0001$

Stool SCFAs demonstrated increases in acetic acid at both time points, along with rising trends in butyric, hexanoic, and propionic acids. Plasma SCFAs followed a similar trend, with acetic acid significantly increasing at week 4, but only trending upward at week 8. Propionic acid trended up in stool but trended down in plasma at week 8, suggesting potential changes in SCFA metabolism or absorption.

Shifts in Hormones

Synbiotic Supplement

After 4 weeks of synbiotic supplementation, the average concentration of estradiol significantly increased from 0.8 to 1.2 pg/ml ($p = 0.05$) and the average concentration of aldosterone trended down from 50.3 to 31.6 pg/ml ($p = 0.16$). After 8 weeks of synbiotic supplementation, the average concentration of DHEAS trended down from 4.2 to 3.5 ng/ml ($p = 0.12$) while progesterone trended down from 26.8 to 10.5 pg/ml ($p = 0.10$). These results are shown in Fig. 8a and b.

Estradiol demonstrated a statistically significant increase early (week 4). Aldosterone, DHEAS, and progesterone all trended down over time, though not reaching statistical significance. Synbiotic supplementation may influence hormone levels, particularly increasing estradiol in the short term. Declining trends in aldosterone, DHEAS, and progesterone all suggest potential effects on adrenal or steroid hormone metabolism, although the results were not statistically significant.

Myoinositol-Based Herbal Supplement

After 4 weeks of supplementation with the myoinositol-based herbal supplement, the average concentration of progesterone trended down

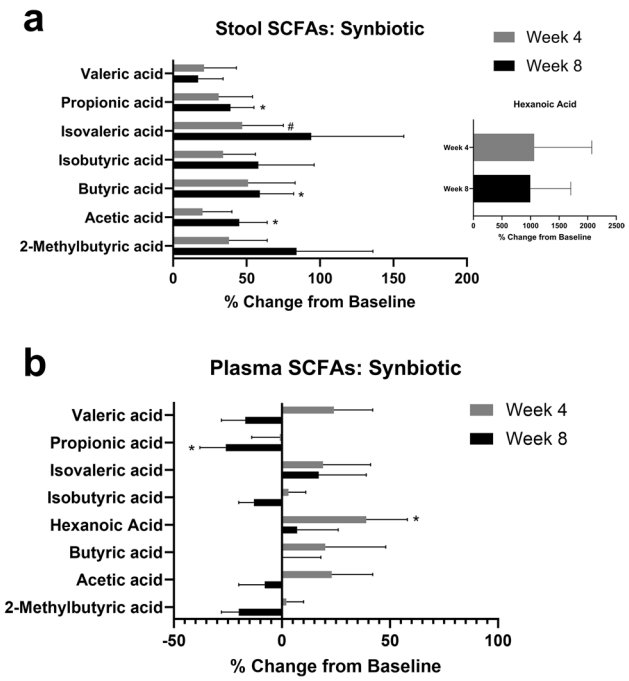

Fig. 6 Percent change in **a** stool and **b** plasma short chain fatty acids in the synbiotic group at week 4 and week 8 compared to baseline. $^{*}p < 0.05$, $^{\#}0.05 > p < 0.2$

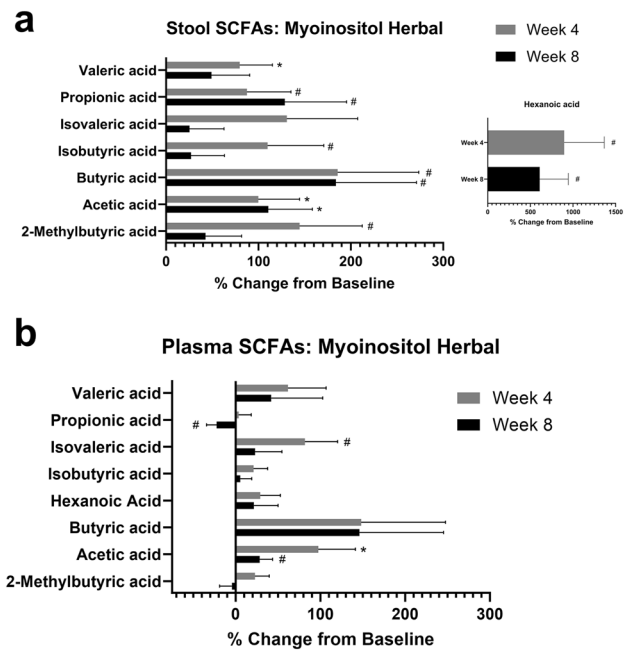

Fig. 7 Percent change in **a** stool and **b** plasma short chain fatty acids in the myoinositol-based herbal supplement group at week 4 and week 8 compared to baseline. $^{*}p < 0.05$, $^{\#}0.05 > p < 0.2$

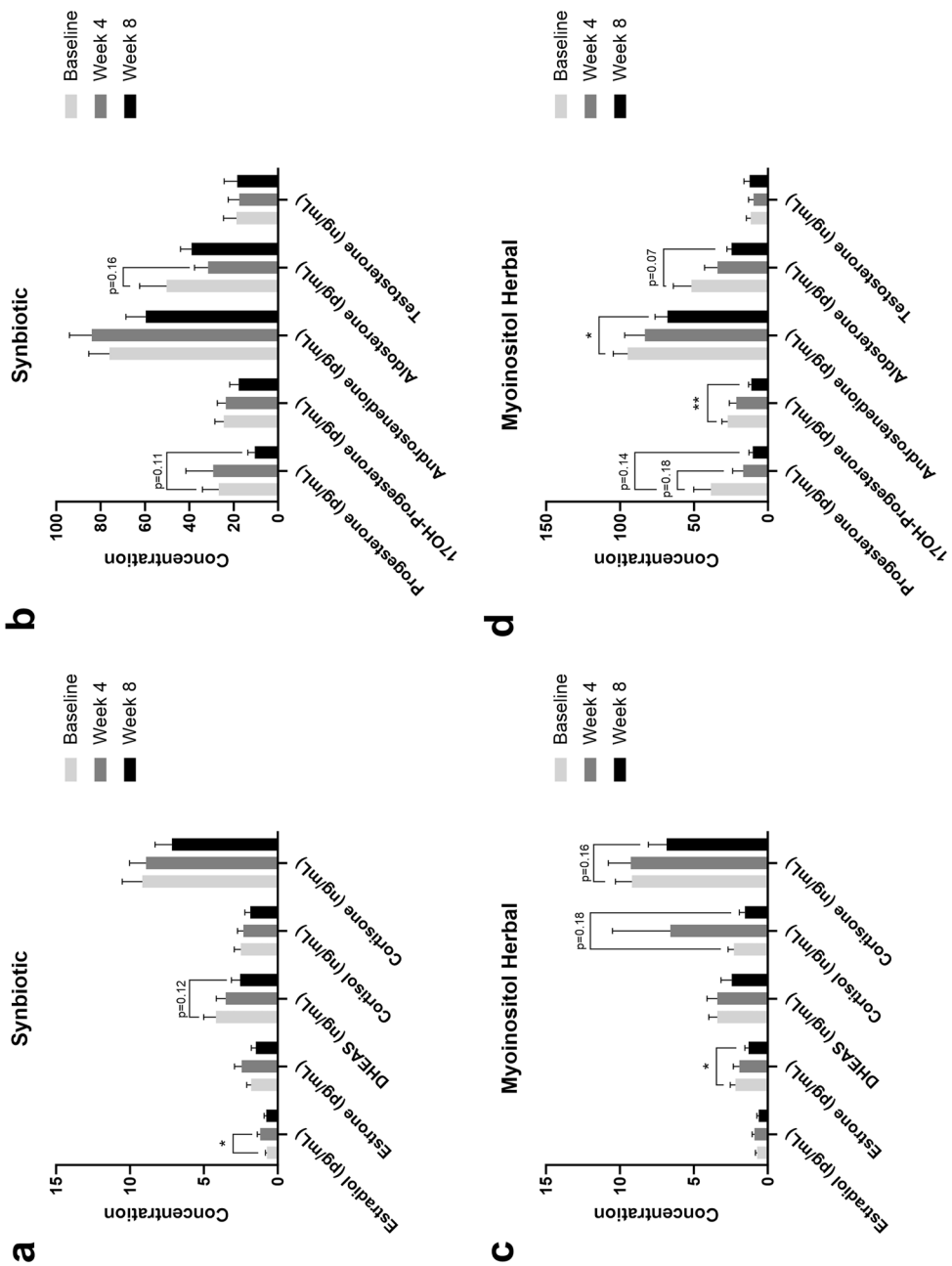

Fig. 8 Concentrations of salivary hormones at baseline, week 4, and week 8 in the **a**, **b** synbiotic and **c**, **d** myoinositol-based herbal supplement groups. $^{*}p < 0.05$, $^{**}p < 0.01$

from 38.6 to 16.8 pg/ml ($p=0.18$). After 8 weeks of supplementation, the average concentration of estrone significantly decreased from 2.2 to 1.3 pg/ml ($p=0.05$), cortisol trended down from 2.3 to 1.6 pg/ml ($p=0.18$), cortisone trended down from 9.2 to 6.8 pg/ml ($p=0.16$), progesterone trended down from 38.6 to 10.3 pg/ml ($p=0.14$), 17-OHP significantly decreased from 27.3 to 11.3 pg/ml ($p=0.001$), androstenedione significantly decreased from 94.9 to 68.0 pg/ml ($p=0.04$), and aldosterone trended down from 51.8 to 24.7 pg/ml ($p=0.07$). These results are shown in Fig. 8c and d.

Significant reductions in estrone, 17-OHP, and androstenedione suggest notable effects on steroid hormone metabolism. Progesterone showed a consistent downward trend across both time points. Cortisol, cortisone, and aldosterone trended down, suggesting possible adrenal effects, but were not statistically significant. Thus, the myoinositol-based herbal supplement may influence hormonal balance, and further studies are needed to better assess potential shifts.

Facial Photography

High-resolution photos were taken of the face for both intervention groups at baseline, week 4, and week 8 (Fig. 9).

Adverse Effects

In the synbiotic group, three subjects experienced adverse effects. One subject with a history of anxiety reported an isolated anxiety episode with palpitations while taking the synbiotic. Another subject withdrew from the study because of an intermittent headache prior to week 8. Finally, one subject experienced a swollen knee during the study; however, this resolved on its own and did not affect their involvement in the study. The anxiety episode and the swollen knee were not believed to be related to the supplement intake. In the myoinositol-based supplement group, three subjects experienced adverse effects. One subject experienced a self-limiting stomachache, reduced appetite, and fever for 4 days. Another subject experienced an itchy and dry throat that resolved on its own. The last subject experienced a mechanical ankle sprain during the study and dropped out of the study prior to week 8. None of these adverse effects in the myoinositol-based supplement group were deemed to be secondary to the supplement.

DISCUSSION

This study shows that a synbiotic supplement and a myoinositol-based herbal supplement both significantly improve non-cystic acne. Their efficacy may potentially be mediated through beneficial shifts in the gut microbiome, augmenting the presence of SCFAs in the stool and the plasma, and modulating hormones that are typically associated with acne. However, the two supplements investigated have different properties and mechanisms of action and this was also demonstrated in the results of the study.

The improvement in acne that we observed in the synbiotic supplement group agrees with previous studies of probiotics [11, 19, 34]. The herbs and phytochemicals utilized in the synbiotic formulation have also been studied for acne previously, such as *Commiphora mukul* [35] and EGCG [36]. However, the novelty in this study is that the supplement combines both probiotics along with herbs and plant-derived ingredients that appear to have a postbiotic effect on the gut. The resulting synbiotic shifted the gut microbiome toward the production of more SCFAs in the stool that also resulted in augmentation of SCFAs in the plasma.

The improvements in acne within the myoinositol-based herbal supplement group suggest an even greater multifactorial response. Although this intervention did not contain probiotics, several key ingredients have been studied for mechanisms driving acne. Myoinositol regulates hormonal influences for acne [23]. *Silybum marianum*-based extracts and its phytochemical silymarin interfere with comedone formation [22] and improve acne [37]. *Ocimum sanctum* L. (holy basil) has been shown to have antimicrobial activity against *C. acnes* [26]. Both

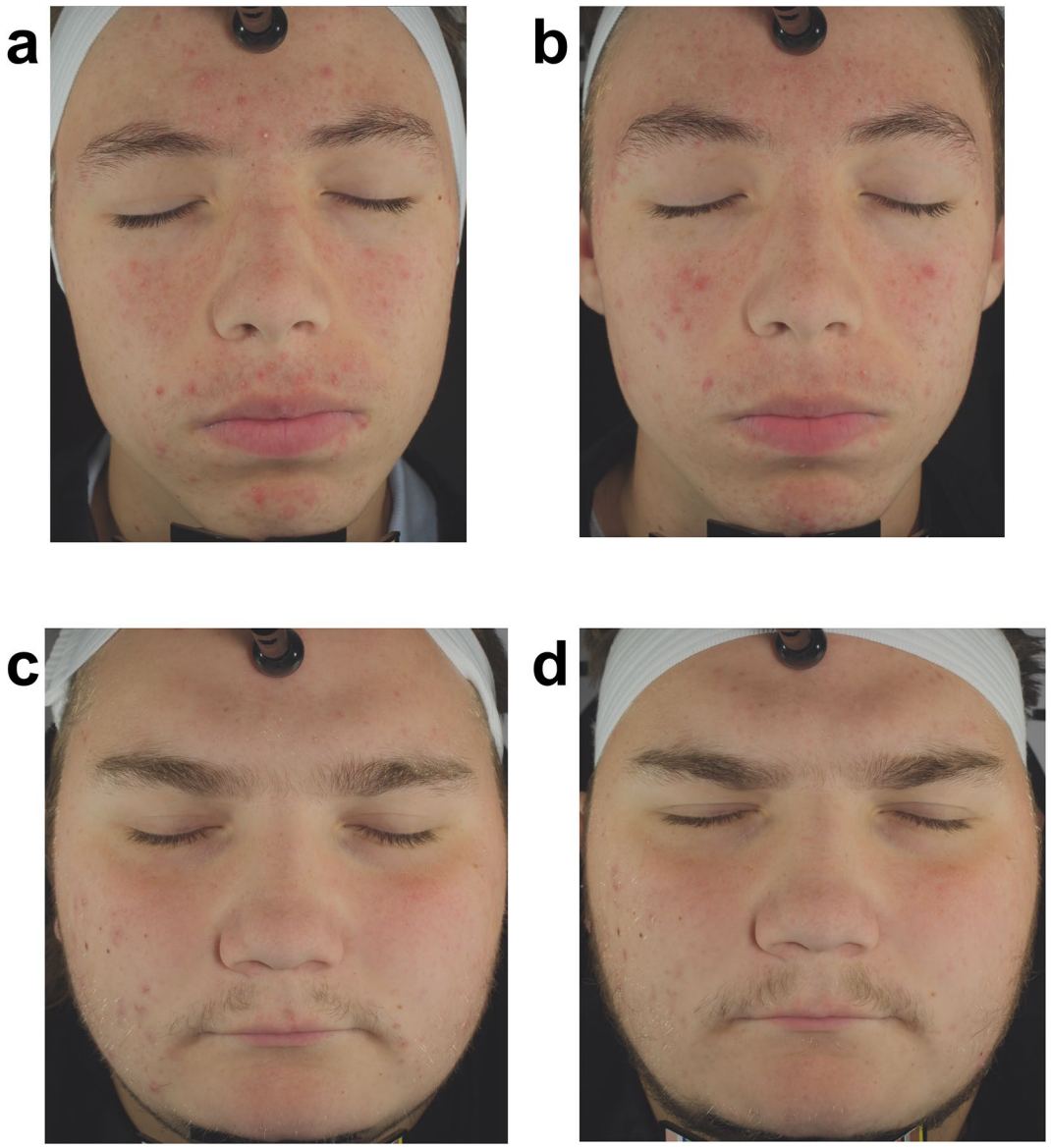

Fig. 9 High-resolution facial photography of a subject in the synbiotic group at **a** baseline and **b** week 8. High-resolution facial photography of a subject in the myoinositol-based herbal supplement group at **c** baseline and **d** week 8

O. sanctum and L-theanine have been shown in randomized controlled studies to improve stress [27, 38]. Our results suggest that the myo-inositol-based herbal supplement has prebiotic activity based on how it shifted the gut microbiome toward the production of more SCFAs, in addition to its effect on hormonal regulation as demonstrated by the changes in 17-OHP and androstenedione levels.

Although there was no change in the Shannon diversity in either treatment group, there was an overall increase in stool and circulating SCFAs for both cohorts which was supported by gut microbiome shifts demonstrating a greater than threefold increase in SCFA-producing bacterial species including *Akkermansia*, *Faecalibacterium*, and *Bifidobacterium*. Increased abundances of SCFA-producing bacteria have been shown to positively influence inflammatory

diseases including cardiometabolic and neurodegenerative disorders, obesity, and notably, inflammatory skin diseases such as acne [5, 39]. The mechanisms by which SCFA-producing bacteria exert benefits for acne are primarily driven by anti-inflammatory effects associated with enhanced immunity, metabolism, and intestinal barrier function driven by SCFAs [5]. For example, *Akkermansia muciniphila* and *Faecalibacterium prausnitzii* have been shown to regulate anti-inflammatory and pro-inflammatory cytokines as well as the NF-κB pathway, thereby demonstrating potential to be a therapeutic target in immune-related and inflammatory diseases [40]. *Bifidobacterium breve* (*B. breve*), one of the bacteria that were increased in this study, acts similarly as an SCFA producer. Moreover, an in vitro study has also shown that *B. breve* may have antibacterial activity against *C. acnes* [41]. When *B. breve* was taken as a part of a dietary supplement containing other probiotics and botanical extracts in a randomized double-blind, placebo-controlled study, there was a significant reduction in the number of inflammatory lesions, decrease in mean desquamation score, sebum production rate, and a decrease in skin abundance of *C. acnes* and *S. aureus* [21]. Given that supplementation with either intervention increased the relative abundance of SCFA-producing bacteria by greater than threefold at 8 weeks, this may be one mechanism driving acne improvement within both groups in our study.

In addition to increasing beneficial bacteria, both cohorts experienced a reduction in pathogenic bacteria at week 8. This reduction may be related to direct competition, where an increased number of beneficial bacteria compete with pathogenic bacteria for nutrients and hinder extensive colonization [42]. Many beneficial bacteria, like *Bifidobacterium*, also produce antimicrobial peptides known as bacteriocins and organic acids that have strong inhibitory effects against pathogens [42]. Furthermore, SCFA-producing bacteria enhance barrier function, thereby preventing pathogens from adhering to the intestinal epithelium, and activate innate and adaptive immune responses that create an inhospitable environment for pathogens [43].

There were several interesting findings regarding the salivary hormone results. Overall, both groups experienced a reduction in androgens or androgen precursors associated with acne pathogenesis. However, after 8 weeks of supplementation with the myoinositol-based herbal supplement, there was a significant decrease in 17-OHP and androstenedione which was not observed in the synbiotic supplement group. Interestingly, one study demonstrated that, in adolescent male patients, the levels of 17-OHP were significantly higher in those with severe acne versus mild acne, potentially elucidating that higher 17-OHP levels increase acne severity [44]. Therefore, a reduction in 17-OHP may correlate with a reduction in acne severity. Moreover, within the androgen production pathway, 17-OHP is converted to androstenedione by the enzyme 17,20-lyase which is then converted to testosterone, a potent androgen, by the enzyme 17β-hydroxysteroid dehydrogenase [45]. Testosterone can then be further converted to dihydrotestosterone (DHT), another potent androgen, by 5α-reductase [45]. Elevated levels of these androgens lead to increased sebum production, follicular hyperkeratosis, and inflammation within the pilosebaceous unit, all of which are driving factors for acne [46]. Thus, one mechanism by which the myoinositol herbal supplement may work on acne is by significantly reducing the precursors to these potent androgens. Further investigation is necessary to understand how individual hormonal profiles may change these findings and whether myoinositol by itself is driving these hormonal changes.

Our findings for hormonal modulation with myoinositol-based herbal supplementation offer a significant advance when considering acne therapies and supplements, especially for male subjects with non-cystic acne. Currently no oral hormone-modulating treatments are available for men as oral spironolactone has unacceptable side effects [10]. However myoinositol at similar or higher doses has been previously studied in men for improving fertility [47] and erectile dysfunction [48] suggesting that it can be supplemented in male individuals safely. In women, myoinositol has been shown to improve metabolic health with improvements in fasting insulin and insulin

resistance in those with polycystic ovarian syndrome [24]. Therefore, myoinositol is a versatile ingredient that may modulate the hormones related to acne in both men and women.

There are several limitations to this study. The duration of this study was 8 weeks and does not address longer-term exposure to the supplements. Regardless, this study was able to establish the multifactorial mechanism of action of both supplements and their ability to improve non-cystic acne. Future studies may consider a longitudinal study design to follow long-term results. This study did not have a control group, although the primary objective of this study was to compare the study interventions against one another. Furthermore, the longitudinal changes in acne lesion counts were associated with improvements in gut health, stool and plasma SCFAs, and shifts in hormones further supporting the clinical results of the study. Expanding on these results with a placebo or control group is warranted. There were no dietary restrictions in this study which may have potential confounding effects, given that certain dietary habits may either worsen or improve acne. However, the subjects were directed to maintain their regular dietary habits without any changes during the study to remove the effect of this potential confounder.

CONCLUSION

The data from this study suggests that both a synbiotic and a myoinositol-based herbal supplement improve non-cystic acne, augment SCFA-producing beneficial bacteria in the gut while reducing pathogens, increase SCFAs in the stool and plasma, and reduce precursors to androgens that drive acne pathogenesis. Notably, the myoinositol-based herbal supplement significantly reduced 17-OHP and androstenedione, potentially elucidating a mechanism by which it reduces potent androgens and regulates the hormonal component of acne. Future prospective studies are warranted that expand on these findings in larger populations and with comparisons against other treatment options such as antibiotics or hormone-modifying agents.

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Data Availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of Interest. Jessica Maloh serves as a consultant and stockholder for Codex Labs. Raja K. Sivamani serves as a scientific advisor and a holder of stock options with Codex Labs, a scientific advisor to Arbonne, and as a consultant to Burt's Bees, Novozymes, Nutrafol, Abbvie, Leo, Galderma, Pfizer, UCB, Incyte, Sanofi, Novartis, Arcutis, Amgen, Sun and Regeneron Pharmaceuticals. Mildred Min, Nasima Afzal, Ajay Dulai, Nabeel Ahmad, and David Pinzauti report no conflict of interest.

Ethics Approval. The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Allendale

Institutional Review Board (CB_Acne_Supp) on June 26, 2023. Written informed consent and assent (where appropriate) was obtained from all subjects and/or guardians involved in the study.

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