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1 **Effect of isotretinoin and omega-3 fatty acid combination on lipid metabolism in**
2 **patients with acne vulgaris: A randomized controlled trial**

3 **Abstract**

4 **Background:** ¹Acne vulgaris (AV) is a chronic inflammatory dermatological
5 condition, and its underlying pathogenesis may be associated with dyslipidemia.
6 Omega-3 fatty acids regulate lipid metabolism and reduce inflammation. However,
7 their effects on blood lipids in AV have not yet been reported. This study's objective is
8 to investigate ¹the serum lipid metabolomic differences between patients and healthy
9 individuals and explore the effects of isotretinoin combined with omega-3 fatty acids
10 on serum lipid metabolism in AV.

11 **Methods:** Between ²July 2020 and June 2021, 46 patients with ¹acne vulgaris and 20
12 healthy controls were recruited from the Affiliated Hospital of Southwest Medical
13 University. ¹Untreated patients were randomly allocated to receive oral isotretinoin
14 combined with or without omega-3 fatty acids for 6 months. Serum lipid
15 metabolomics was performed using liquid chromatography-tandem mass
16 spectrometry (LC-MS). Statistical analysis of the lipid metabolites was conducted by
17 partial least squares-discriminant analysis (PLS-DA). Correlations between
18 differential lipid metabolites and disease severity indicators were tested by
19 Spearman's correlation analysis. Metabolic pathways in the isotretinoin and omega-3
20 combination treatment ¹group were analyzed using the Kyoto Encyclopedia of Genes
21 and Genomes (KEGG) database.

22 **Results:** PLS-DA showed a clear separation of serum lipid metabolites between
23 healthy controls and AV patients, with the combination treatment group showing a¹
24 trend towards the healthy controls. The lipid metabolite composition of the
25 combination treatment group showed significant changes, with 245 differential
26 metabolites identified before and after treatment ($P < 0.05$). The Spearman's
27 coefficient showed a strong correlation between the serum level of StE (20:5) ($6.44 \pm$
28 0.33 vs. 7.18 ± 0.22 , $P < 0.001$) and the Global Acne Grading System score ($R = 0.73$,
29 $P < 0.001$). The KEGG analysis identified 18 different pathways before and after
30 treatment in the combination treatment group.

31 **Conclusions:** We found²⁸ that omega-3 fatty acid supplementation improved the
32 clinical responses in AV patients, correlating with improved lipid metabolism, thereby
33 confirming its potential as a valuable adjunct therapeutic strategy.

34 **Trial registration:** The comprehensive¹ clinical trial protocol has been
35 retrospectively registered on the ISRCTN Registry of Clinical Trials website (NO.
36 ISRCTN31758284) on 2022/07/09.

37 **Keywords:** Acne vulgaris, Lipid metabolism, Isotretinoin, Omega-3 fatty acids,
38 Combined Modality Therapy

39

40 ¹**Background**

41

42 Acne vulgaris (AV) is a chronic inflammatory condition that primarily affects the
43 sebaceous gland units ranks the eighth most frequent cutaneous disorder with a global
44 morbidity of up to 9.38% [1]. Traditionally, the pathogenesis of AV is thought to
45 involve ¹multiple factors, including androgen-mediated increased sebum secretion,
46 altered keratinization, inflammation, and *Cutibacterium acnes* colonization [2].
47 Several investigations have suggested that dysregulation of serum lipid metabolism
48 represents a contributing factor in the onset and advancement of AV condition,
49 including dyslipidemia, such as ¹⁵elevated levels of serum low-density lipoprotein
50 cholesterol (LDL-C), triglyceride (TG), cholesterol (TC) ¹and free fatty acids (FFA)
51 [3]. Despite it being a major global health issue, existing treatment options for AV are
52 limited by adverse reactions, making drug safety and efficacy the primary
53 considerations in clinical decisions. The existing traditional treatments, such as using
54 isotretinoin, have been confirmed to cause side effects, including cheilitis, dryness,
55 photosensitivity, teratogenicity, and depressive mood [4]. Additionally, research on the
56 utility of monitored isotretinoin intervention for AV has revealed that patients may
57 experience dyslipidemia, such as hypertriglyceridemia, even during treatment [5].
58 Therefore, safer therapies and adjuvant medications are urgently required for patients
59 with AV.

60 ²⁶Omega-3 (ω -3) fatty acids belongs to the polyunsaturated category ¹often obtained
61 from marine fish and certain plant oils [6]. Compelling evidence suggests that ω -3
62 fatty acids, owing to its anti-inflammatory capability, offers a promising new
63 therapeutic approach for inflammatory skin conditions, including psoriasis and atopic

64 dermatitis [7,8]. Moreover, its ¹well-recognized efficacy against AV has been
65 reinforced by positive outcomes in clinical trials [9]. Concurrently, the capacity ²of ω
66 -3 fatty acids to reduce lipid levels has ¹attracted widespread attention in populations
67 with overweight individuals or those with hyperlipidemia [10,11]. Despite
68 recognizing both anti-acne and lipid-modulating capability ²of ω -3 fatty acids, whether
69 its lipid-regulating capacity contributes to its therapeutic efficacy in AV remains
70 unexplored. Previous studies had not explored the potential ¹of ω -3 fatty acids on the
71 serum lipid profile thoroughly, specifically in AV.

72 Therefore, the current randomized controlled trial (RCT) aimed to assess the
73 benefit and potential mechanisms of the ¹combination of isotretinoin and ω -3 fatty
74 acids intervention in clinical efficacy and ²serum lipid metabolites in AV patients. The
75 findings provided a mechanistic rationale to support ω -3 fatty acid supplementation
76 acts a novel and safe adjunctive strategy for AV management.

77

78 **Methods**

79 ¹1. Population

80

81 Prior to the study, all participants allowed ¹⁷the use of data and samples for scientific
82 purposes and signed written informed consent form. ¹From July 2020 to June 2021, 46
83 patients diagnosed with moderate-to-severe AV, matched along with 20 healthy
84 controls (the HC group) were enrolled in the study ³from the dermatology department

85 of the Affiliated Hospital of Southwest Medical University. Disease²⁴ severity was
86 determined by the Global Acne Grading System (GAGS) score, with GAGS $\geq$ 19
87 defined as moderate to severe AV [12]. Beyond adhering to the criteria established in
88 prior study [13], the participants who had already received intervention of isotretinoin,
89 antibiotics, glucocorticoids,¹ ω -3 fatty acids, immunosuppressive drugs, or herbal
90 treatments within the past 6 months, or were unwilling to sign the informed consent
91 form, or were known to be allergic to the intervention were all excluded.¹ Moreover,
92 daily lifestyle changes were recommended during the treatment, including less sweet
93 intake, a high-fat diet, and dairy products. The Ethics Committee had given¹ approval
94 for the use of human subjects in this study¹ (No. KY2020115).

95 2. Clinical trial design

96
97 The patients were randomized to receive orally taken isotretinoin treatment
98 (Huabang Pharmaceutical Co., Ltd. Chongqing, China), either combined with (the
99 AVW group) 2400 mg/day¹ ω -3 fatty acids (Nature Made, LA, USA) or monotonously
100 (the AVP¹ group) for 6 months. When the medicines were administered to patients,
101 identifiable packaging was removed so that the patients could not know the name of
102 the medicine they were taking. The isotretinoin dosage¹ was adjusted based on drug
103 tolerance and treatment efficacy. Therapeutic effects were evaluated using the GAGS
104 score.¹ When GAGS score was between 19 and 30, the isotretinoin dose was set at 0.5
105 mg/ kg/day; when the score exceeded 30,¹ the dose was applied to 1.0 mg/ kg/day.
106 Demographic data of the participants, including sex, age, body mass index (BMI), and

107 disease duration were recorded at enrollment. GAGS score evaluation, skin analysis
108 by vital imaging system for aesthetic analysis (VISIA), and blood serum sample were
109 collected²³ at baseline and at the end of treatment. The study¹ used the CONSORT
110 reporting guidelines to draft this manuscript and the CONSORT reporting checklist
111 for editing¹ [14].

112 3. Efficacy evaluation criteria and VISIA

113

114 GAGS divides acne-prone areas into the nose and chin area (1 point),² forehead (2
115 points), left cheek (2 points), right cheek (2 points), and chest and upper back (3
116 points) regions, based on the approximate area and density¹ of the pilosebaceous units.
117 Different zones correspond to different weight scores,¹ 1–18 points for mild, 19–30
118 points for moderate, 31–38 points for severe, and ≥ 39 points for extremely severe.
119 Only moderate to severe cases were enrolled in this study.

120 A⁷ VISIA-CRTM imaging system (Canfield Scientific, Inc., USA) was used to
121 evaluate the average percentage of facial red and purple areas of the participants¹
122 before and after treatment. The red area index reflects the condition of capillaries in
123 patients with AV, whose blood vessels and hemoglobin in the dermal papilla layer of
124 the skin appear reddish. On the other hand, the purple area index measures porphyrins,
125 which are metabolites of *Propionibacterium acnes* at the hair follicle opening and
126 appear as brick-red highlights. A higher percentage of VISIA indices indicates milder
127 disease severity.

128 4. Sample collection and detection

129

130 Serum were collected from 2 mL of venous blood at fasting in non-anticoagulant
131 tubes at the beginning of enrollment and the end of their 6-month treatment,¹ samples
132 were centrifuged after collection at 3,000 rpm for 15 min after 30 min of resting at
133 20–25 °C, and subsequently stored in a -80 °C refrigerator. An automated biochemical
134 detector (Synchron LX-20; Beckman Coulter, USA) was used to determine the blood
135 glucose (Glu), TC, TG,³ LDL-C, and high-density lipoprotein cholesterol (HDL-C)
136 levels.¹² Insulin resistance was assessed by homeostasis model assessment of insulin
137 resistance (HOMA-IR). Fasting insulin (INS) levels were measured by the ELISA kit
138 (Promega, USA).

139 ¹5. Serum sample processing for lipidomics analysis

140

141 For lipid extraction, 200 µl of serum samples, 80 µl of methanol (Cat: M742213,
142 Macklin, Shanghai, China)⁷ and 400 µl of methyl-tert-butyl ether (MTBE, Cat:
143 B802533, Macklin, Shanghai, China) were mixed; the mixtures were vortexed⁸ for 30
144 s and then ultrasonically extracted for 30 min on 40 kHz power at 5 °C.¹ The samples
145 were settled at -20 °C for 30 min then centrifuged (13000 rpm, 4 °C) for 15 min. Next,
146 supernatant was drawn out and dried in a vacuum concentrator (Cat: Y-TN-C19819,
147 Yuyan Machinery Co., LTD, Shanghai, China), and 100 µl of isopropanol (Cat:
148 I766839, Macklin, Shanghai, China):acetonitrile (1:1, v/v, Cat: A801556, Macklin,

Shanghai, China) solution was added¹ for re-dissolution. After 30 s of vortexing, the mixtures were ultrasonically extracted in ice water bath and centrifuged (13000 rpm, 4 °C) for 5 min then transfer 80 µl of supernatant to¹ UPLC-Q Exactive System (Thermo, CA).

6. Acquisition of LC-MS/MS data

The study obtained metabolites were processed by an Ultra High Performance Liquid Chromatography Tandem Fourier Transform Mass Spectrometry UPLC-Q Exactive system (Thermo Scientific™ Vanquish™ UHPLC, CA). Two microliters⁸ of the sample were separated by a BEH C18 column (0.1 cm × 0.0021 cm¹ i.d., 1.7 µm) and then operated by mass spectrometry. Solvent A, which contained 0.1% formic acid (Catalog: F809715, Macklin, Shanghai, China) dissolved in 50% acetonitrile,³ 10 mmol/L ammonium acetate solution; solvent B, which consisted of¹⁸ 2 mmol/L ammonium and 0.02% formic acid in acetate solution mixed with isopropanol, acetonitrile and water in a volumetric ratio of 10:88:2, these two distinct solvent systems in which¹⁹ the mobile phase was composed. The solvent gradient elution protocol was established as follows: an initial composition of 65% solvent A and 35% solvent B was maintained from 0 to 4 minutes, transitioning to 40% A and 60% B. Subsequently, from 4 to 12 minutes, the gradient shifted from 40% A:60% B to 15% A:85% B. Between 12 and 21 minutes, the composition was further adjusted from 15% A:85% B to 0% A:100% B. A rapid re-equilibration step occurred from 24.0 to 24.1 minutes, returning the mobile phase to 65% A and 35% B. Finally, from 24.1 to 28

171 minutes, the system was held isocratically at 65% solvent A and 35% solvent B to
172 ensure column reconditioning. Chromatographic separation was performed⁵ with a
173 constant flow rate of 0.40 mL/min and a column temperature maintained at 40°C. For
174 mass spectrometric detection, signals were acquired in both positive and negative
175 ionization modes simultaneously. The operational parameters were configured under
176 mass²⁰ scan range (m/z), 200-2000, spray voltage, 3.00 kV or both modes;³⁰ ion source
177 temperature, 370 °C, collision energy, 20–60 V.

178 ¹ 7. Data process and statistical analysis

179

180 The raw LC-MS data were imported into LipidSearch (Thermo Fisher Scientific)
181 ¹ software to obtain a data matrix for subsequent analysis. Multivariate statistical
182 analyses were applied³ from Bioconductor on the Majorbio Cloud Platform
183 (<https://cloud.majorbio.com>) utilizing the R programming environment⁵ (version 4.1.1).
184 Partial least squares-discriminant analysis (PLS-DA) or orthogonal partial least
185 squares-discriminant analysis (OPLS-DA) was employed to provide a comprehensive
186 view of the metabolic dataset, facilitating the assessment of overall clustering patterns,
187 discernible trends, and identification of potential¹ outliers. Volcano plot analysis of
188 differential expression provided dual validation (P -value+ FC), emphasizing
189 statistical significance, magnitude of biological change, and the direction of changes
190 before and after treatment. The metabolites of significantly difference were selected
191 out on the basis of¹ the variable weight value ($VIP \geq 1$, fold change ($FC > 1$ / $FC < 1$
192 and P value < 0.05). The biochemical pathways and associated metabolic processes

193 were identified³¹ through metabolic enrichment analysis and pathway mapping by the
194 ¹Kyoto Encyclopedia of Genes and Genomes (KEGG) database (kegg_v2021.09.18)
195 and Scipy.stats (Python package) (<https://docs.scipy.org/doc/scipy/>) to identify those
196 significantly enriched pathways.

197 8. Statistical analysis 用 means $\pm$ standard deviation

198

199 ⁸All statistical analyses were conducted by the SPSS (version 25.0). Quantitative
200 data are presented as means $\pm$ standard deviation. This study employed ⁷the
201 Mann-Whitney U test to assess the differences in the data both between¹ and within the
202 two groups, the Wilcoxon test to appraise the intervention before and after within the
203 same group, the Spearman's rank correlation coefficient to estimate the monotonic
204 correlation between variables. Statistical significance standard was ¹⁰set at $P < 0.05$.
205 GraphPad Prism (version 8.0) was employed for data visualization.

206

207 Results

208

209 1. Background and efficacy of combination therapy in patients with AV

210

211 ¹Three patients withdrew from the AVP group due to nosebleeds, generalized
212 eczema, and severely chapped lips, and three were lost to follow-up in the AVW

213 group. Twenty patients were eventually included in each group for the clinical
214 analysis (Supplement. 1).¹⁴ The baseline demographic and clinical characteristics are
215 exhibited in Table 1. The differences in baseline data among the three groups in terms
216 ¹ of gender, BMI, HOMA-IR, INS, HDL-C, LDL-C, TG, and TC were not statistically
217 significant ($P_1 > 0.05$).³ While there was an age difference existed in the HC group and
218 the patient group ($P_1 < 0.001$), all individuals enrolled in the study were within the
219 18 – 30 age range specified by the inclusion criteria.¹ Regardless of the application of
220 ω -3 fatty acids, the intervention group showed varying degrees of improvement in
221 facial GAGS scores ($P_2 < 0.001$, $P_3 < 0.001$), sclererythrin levels (²⁵ $P_2 < 0.001$, $P_3 <$
222 0.001) and percentage of red areas ($P_2 < 0.001$, $P_3 = 0.006$) after treatment. More
223 importantly, the AVW group achieved a significantly greater improvement in GAGS
224 scores ($P_4 = 0.023$ for AVP1 vs. AVW1) and TG levels ($P_4 = 0.01$ for AVP1 vs. AVW1)
225 than the AVP group.

226 ¹ 2. Effect of ω -3 fatty acids on lipid metabolomics composition in patients with AV

227

228 Lipid metabolism was investigated to explore the effect of ω -3 fatty acids on serum
229 lipid metabolomics composition, a total of 1021 quantifiable peaks were detected
230 among the 60 serum samples. Due to lipid extraction failure, a total of five samples
231 were excluded, two from the HC group, two from the AVP group, and one from the
232 AVW group,¹ and 258 lipid metabolites were finally measured after removing all false
233 positives and noise data. A PLS-DA model was applied on the complete dataset to
234 estimat the clustering tendency from the patients before and after treatment and the

235 HC group. As shown in Figure 1A,¹ the separation trend of metabolite compositions
236 across the HC group and the patients in both AVP0 and AVW0 groups was clearly
237 observed at baseline, especially in the cation model (Figure 1A), suggesting that the
238 lipid metabolite structures in AV patients differ from those in HC. Following
239 intervention, the lipid metabolism profiles of both AVW1 and AVP1 groups drifted
240 closer to that of the HC group (Figure 1C, D). However, the drift was more
241 pronounced in AVW1 group, rather than AVP1 group, a disparity more evident in the
242 cation model (Figure 1C). Further permutation tests for¹ the anion and cation models
243 established by PLS-DA showed that the models had no overfitting problem and had
244 good reliability and validity (Figure 1E-H).

245 A clear separation between the AVW1 and AVP1 groups in both cation and anion
246 models was confirmed using OPLS-DA, further validating the distinct effects of the
247 two interventions (Supplement2. A-D). Permutation tests for the OPLS-DA models
248 also demonstrated¹ no overfitting, and indicated good reliability and validity
249 (Supplement2. E-H).

250 3. Combination therapy altered² serum lipid metabolites of the patients with AV

251

252 Volcano plots were used¹ to examine the composition of differential lipid
253 metabolites. This provided double validation of the previous results and emphasized
254 the direction of variation of the metabolites² after ω -3 fatty acid treatment. As shown
255 in Figure 2A, B,¹ the metabolites formed two independent clusters; 79 and 54

256 upregulated metabolites and 83 and 29 downregulated metabolites were observed in
257 the cation and anion models, respectively. Overall, 509 and 267 metabolites showed
258 no significant change.

259 ¹A total of 245 lipid metabolites with significant differences between AVW0 and
260 AVW1 ($P < 0.05$) were identified. With P value ¹ < 0.05 , $FC > 1$ or $FC < 1$ and $VIP \geq 1$
261 set as the screening conditions, Table 2 delivers the top 30 lipid metabolites exhibiting
262 the most significant differences between groups. The ¹levels of one type of
263 acylcarnitine (AcCa), phosphatidylcholine (PC), sphingomyelin (SM), one type of
264 sulfoquinovosyl (SQDG), and two types of diacylglycerol (DG) were elevated in the
265 AV group when compared to the HC; however, they ¹showed a certain degree of
266 decrease after ω -3 fatty acid treatment, approaching the levels of the HC ($P < 0.05$).
267 Conversely, the levels of one type of cardiolipin (CL), bis-methyl phosphatidic acid
268 (BisMePA), hexosyl ceramide (Hex2Cer), lysodimethylphosphatidylethanolamine
269 (LdMePE), lysophosphatidylcholine (LPC), eicosapentaenoic acid ethyl ester (StE),
270 ¹Zymosterol (ZyE), two types of diacylglycerol (DG) and phosphatidyl ethanolamine
271 (PE), three types of phosphatidylcholine (PC), and nine types of TG were decreased
272 in the AV groups than in the HC; the levels increased to almost that of HC after ω -3
273 fatty acid treatment ($P < 0.05$). Overall, the ω -3 fatty acid and isotretinoin
274 intervention ¹had an impact on lipid metabolism in AV patients.

275 ¹4. Correlation analysis of differential lipid metabolites and therapeutic efficacy

276

277 The heatmap shows ¹significantly different lipid metabolites and their main
278 biological functions before and after the combined treatment with isotretinoin and ω -3
279 fatty acids (Figure 3). Correlation analysis indicated that metabolites located in the
280 upper region exhibited a negative association ¹with the GAGS score, that
281 downregulation of their expression could have potential positive therapeutic effects on
282 patients with AV and vice versa; metabolites in the lower part were positively
283 correlated with the GAGS score, that upregulation of their expression could have
284 potential negative therapeutic effects on patients with AV. Next, Spearman's analysis
285 was conducted between the metabolites listed in Table 2 and the GAGS score, which
286 denotes the severity of acne. Among the metabolites, strong co-expression was
287 observed between the serum level of StE (20:5) and the GAGS score ($R = 0.73$, $P <$
288 0.001) while the remaining metabolites were moderately correlated (Figure 3).
289 Meanwhile, the serum level of StE (20:5) in AV patients decreased compared to that
290 in HC ($P < 0.001$), suggesting that it could be a key substance for the intervention of
291 ω -3 fatty acids in lipid metabolites (Table 2).

292 5. KEGG pathway enrichment of metabolites altered by combination therapy

293

294 To investigate the functional characteristics associated with ω -3 fatty acid
295 intervention in AV patients, KEGG enrichment of ²lipid metabolites differentiating the
296 AVW0 and AVW1 groups. Finally, 18 ¹pathways with significant differences were
297 identified, namely the upregulated glycerophospholipid metabolism, alpha-linolenic
298 acid metabolism, retrograde endocannabinoid signaling, ¹linoleic acid metabolism,

299 arachidonic acid (AA) metabolism, diabetic cardiomyopathy, choline metabolism in
300 cancer, and amoebiasis pathways; all the rest were downregulated (Figure 4). Among
301 them, the glycerophospholipid metabolism pathway was the most significantly
302 enriched, with the largest number of differentially expressed molecules ($P < 0.001$).

303

304 Discussion

305

306 Previous studies had confirmed the presence of lipid metabolism disorders in
307 patients with AV. Comparative analysis and in-vitro epidermal models have shown
308 that patients with AV have higher skin fatty acid levels and elevated serum TC, TG,
309 and LDL-C levels than healthy individuals [15]. However, the lipid metabolism
310 disorder may exacerbate¹ inflammation [16]. Specifically, excessive unsaturated fatty
311 acids (UFAs) in sebum are decomposed by bacteria to produce pro-inflammatory
312 lipids such as leukotriene B4 (LTB4), which might lead to skin barrier damage and
313 subsequent thickening of the hair follicle wall and stratum corneum, resulting in
314 inflammation [17].

315 Oral isotretinoin, a classic traditional therapy for treating moderate to severe AV,
316 has been reported to cause dose-related adverse reactions, such as dyslipidemia,
317 manifesting with an increase in serum TG, stimulates the production and release²¹ of
318 very low-density lipoprotein cholesterol (VLDL-C), leading to elevated levels of
319 ²LDL-C and apolipoprotein B (apoB), alongside a reduction in HDL-C.[18].

320 Consistent with these findings, although isotretinoin treatment alleviates disease
321 severity, differences still exist in serum lipidomics between healthy controls and
322 patients with AV after treatment. This suggests that the therapy may not fully recover
323 the systemic metabolic disturbances. Even after the skin lesions are alleviated,
324 patients might retain an altered lipid metabolic state induced by the drug itself, a
325 phenomenon that occurs during drug-induced persistent metabolic states and tolerance
326 [19]. This persistent dysregulation could partly explain disease recurrence in some
327 patients after treatment cessation as well as the observed metabolic side effects
328 associated with long-term use, such as dyslipidemia and insulin resistance [20].
329 However, persistent abnormalities in the lipidomic profile following systemic
330 treatment might provide a molecular basis for understanding the potential metabolic
331 risks associated with isotretinoin use.

332 Notably, the ¹effect of ω -3 fatty acids has received widespread attention, being
333 capable of promoting ¹the clearance of VLDL-C by reducing its synthesis and
334 secretion in the liver, thereby lowering serum TG levels and increasing HDL-C
335 content, which is believed to alleviate the ¹lipid metabolism disorders [21]. In rat
336 models, the levels of blood TG, TC, and LDL-C in the ω -3 fatty acid supplementation
337 group were significantly decreased [22]. The absence of dramatic fluctuations in
338 serum lipid levels post intervention could be attributed to a potential linear
339 relationship between the ω -3 intervention duration and its metabolic effects [23]. An
340 ²obvious regulatory effect was observed in the subsequent lipid metabolomic analysis.
341 Stronger evidence suggested ¹that ω -3 fatty acids have a lipid-decreasing effect in

342 other metabolic diseases. The ω -3 fatty acids supplementation in nonalcoholic fatty
343 liver disease (NAFLD) has confirmed an association with reduced¹ gamma-glutamyl
344 transpeptidase (GGT) activity, decreased liver fat in weight loss individuals, and
345 amelioration in plasma lipid profiles; in a meta-analysis investigating the potential of
346 ω -3 fatty acids in¹ gestational diabetes treatment, ω -3 fatty acid supplements were
347 validated to reduce TG and VLDL-C and increased¹ HDL-C while lowering fasting
348 plasma glucose, levels of inflammatory factors, and reducing insulin resistance
349 [24,25]. Both published literature and the current study supported that ω -3 fatty acid
350 supplementation is beneficial for correcting lipid metabolism disorders in AV.

351 The current study revealed that the metabolic profiles of both intervention groups
352 were similar to those of healthy controls in the PLS-DA score plot, indicating that the
353 interventions effectively modulated the lipid metabolism in AV patients. Notably, the
354 metabolic profiles of the two interventions exhibited modest separation, particularly
355 in the anion model. Lipid metabolism is a conserved biological pathway, and
356 therapeutic interventions typically induce targeted modulation of certain lipid
357 substances rather than a drastic metabolic reprogramming [26]. Since isotretinoin was
358 applied in both interventions, its metabolic effects may have partially overshadowed
359 the modifications conferred by ω -3 fatty acids, making the PLS-DA model exhibit
360 limited distinction between the two groups. Despite the modest separation, a
361 permutation test effectively confirmed the validity of the PLS-DA model, excluding
362 the possibility of random separation. Subsequent OPLS-DA filtered out intragroup
363 unrelated variations, clearly demonstrating a significant separation between the AVP

364 and AVW groups. This confirmed that the² isotretinoin and ω -3 fatty acid combination
365 therapy indeed introduced beneficial metabolic moderation.

366 ¹Following ω -3 fatty acid supplementation, DG(18:1/22:6) and DG(20:5/18:2)
367 exhibited pronounced differences and displayed a clear trend towards normalization.
368 Both the DG species are long-chain molecules, with the former containing a
369 docosahexaenoic acid chain (DHA, 22:6) and the latter containing an
370 eicosapentaenoic acid chain (EPA, 20:5).¹Through which ω -3 fatty acids exert
371 therapeutic benefits are the main components DHA and EPA; previous study had
372 ¹indicated that they could significantly ameliorate lipid metabolism not only by
373 reducing serum and hepatic TG and TC levels and inhibiting lipid accumulation via
374 suppression of fatty acid synthase activity, but also by promoting lipid homeostasis
375 and anti-inflammatory processes through modulation of the PPAR pathway and
376 expression of related genes such as FADS2 [27,28]. Inflammation is well known to
377 serve¹ as a significant trigger for hyperkeratinization of the pilosebaceous duct [29].
378 Although EPA lacks direct antimicrobial activity, its anti-inflammatory character and
379 ability to modulate the sebaceous microenvironment could¹ indirectly influence the
380 proliferation of *Cutibacterium acne* and its capacity to induce inflammatory responses,
381 thereby reducing inflammation triggered by bacterial metabolites and correcting
382 keratinization and the microbial environment [30]. Furthermore, as a precursor for the
383 synthesis of specialized pro-resolving mediators (SPMs), EPA is a potent signaling
384 molecule in the natural resolution of inflammation, capable of suppressing the
385 migration and activation of pro-inflammatory cell types, including neutrophils, while

386 promoting macrophage phagocytosis and apoptotic cells and debris' clearance [31].
387 EPA acts as a competitor, vying with pro-inflammatory substrates like arachidonic
388 acid, a ω -6 fatty acids, for accessing the enzymes ¹cyclooxygenase (COX) and
389 lipoyxygenase (LOX) [32]. This type of competition ultimately reduces
390 pro-inflammatory product, such as prostaglandin E2 (PGE2), LTB4, and
391 thromboxanes, and increases that ¹of other lipid mediators, such as PGE3 and LTB5,
392 which exhibit significantly lower pro-inflammatory effects than PGE2 and LTB4 [33].

393 Notably, StE (20:5) was the only product that demonstrated a strong correlation
394 with the GAGS score. This compound refers to eicosapentaenoic acid ethyl ester (EPA
395 ethyl ester), an anti-inflammatory polyunsaturated fatty acid ¹conferred by its EPA
396 structure that can directly address critical therapeutic targets in acne management.
397 Large-scale clinical trials specifically investigating pharmaceutical-grade EPA ethyl
398 ester, an oral formulation found in prescription medications like Lovaza and Vascepa,
399 were previously conducted for TG reduction in AV treatment [34]. In the current study
400 on moderate-to-severe AV, patients receiving combined ω -3 fatty acid therapy
401 exhibited significantly improved trends in lipid metabolomics and demonstrated
402 greater improvement in severity indices compared to the isotretinoin monotherapy
403 group. This aligned with the findings from other studies that reported reductions in
404 both inflammatory and non-inflammatory diseases through fish oil supplementation or
405 high-fish diets [35].

406 The superior clinical efficacy of the combination treatment invited a deeper
407 interpretation of the relationship between systemic lipid metabolism and acne

408 resolution. While the OPLS-DA model confirmed a distinct lipidomic signature for
409 the combination therapy, and metabolites such as StE (20:5) had strong correlation
410 with clinical severity ($R = -0.73$), the serum lipid profile as a whole may not serve as
411 a precise, linear biomarker for an individual acne lesion. The therapeutic effect of
412 systemic isotretinoin intervention is rooted in the targeting of the pilosebaceous unit,
413 leading to a distinct reduction in sebum production and modulation of follicular
414 keratinization [36]. Therefore, a significant decrease in GAGS scores is a direct
415 reflection of the improvement in local microenvironment [12]. Shifts in the serum
416 lipidome represent systemic metabolic adjustments, which offer a valuable window
417 into the overall metabolic state and treatment-related biochemical shifts. While being
418 informative, they have limitations as a standalone biomarker for tracking acne
419 evolution during therapy. Future investigations aimed at identifying more robust
420 biomarkers should consider including the analysis of pilosebaceous unit lipidomics to
421 better capture the direct therapeutic effects on the target tissue while exploring the
422 metabolic pathways and mechanisms involved.

423 ¹In addition, KEGG enrichment pathway analysis indicated that ω -3 fatty acid is
424 involved in lipid metabolism-related pathways. Glycerophospholipid metabolism, a
425 classic lipid metabolism pathway, is related to the properties of sebaceous gland cell
426 membranes and sebum components [37]. The stability of this metabolic pathway
427 contributes to the preservation of ¹skin barrier and the normal differentiation of
428 keratinocytes at the hair follicle orifice; the latter is a key step in hair follicle blockage
429 at the onset of AV [38]. Moreover, ¹glycerophospholipid metabolism may affect the

430 generation of substances such as prostaglandins and leukotrienes, pro-inflammatory
431 mediators, thereby indirectly exerting anti-inflammatory effects that are closely
432 related to the pathological mechanism of AV [39]. Therefore, the intervention of ω -3
433 fatty acids on the glycerophospholipid metabolism pathway may become a potential
434 research direction for regulating sebaceous gland function and improving hair follicle
435 hyperkeratosis in patients with AV.

436 Substantial research has revealed that combining isotretinoin with other therapies
437 yields synergistic effects, enhances efficacy, and significantly improves safety [40].
438 An RCT employing a combination of desloratadine and isotretinoin demonstrated
439 superior clinical outcomes, lower recurrence rates, and more tolerable adverse events
440 compared to monotherapy with isotretinoin alone [41]. Same combination in another
441 comparative study showed that the benefits of a lower dose of isotretinoin (0.25
442 mg/kg) were equivalent to those of the standard dosage (0.25 mg/kg) [42], and
443 co-therapy with cyproterone could even effectively mitigate isotretinoin-induced
444 dyslipidemia [43]. Studies are not only limited to the optimization of drugs;
445 adjunctive physical treatments such as 675-nm laser therapy, when combined with
446 isotretinoin, can lead to faster and pronounced clinical improvement compared to that
447 in the laser-only group [44].

448 **Strengths and Limitations**

449

450 The study presented the first RCT that evaluated the efficacy of ω -3 fatty acid and
 451 isotretinoin combination therapy on the serum lipid metabolome in AV patients,
 452 providing novel insights that contribute to the understanding of this combination
 453 regimen's potential mechanisms of action. However, the generalizability of these
 454 results and the evaluation of long-term effects may be constrained by the study's
 455 modest sample size. Although the observed alterations in the serum lipid metabolome
 456 reflect systemic metabolic adjustments, the changes may not precisely correspond to
 457 the therapeutic effects within the pilosebaceous unit, which is the primary site of acne
 458 pathology. Future investigations incorporating direct lipidomic profiling of target
 459 tissues are warranted.

460 Conclusions

461

462 In conclusion, ω -3 fatty acid supplementation can positively modulate serum
 463 lipid metabolites in patients with AV, resulting in improved therapeutic responses.
 464 Consequently, the nutritional intervention should be integrated into management as a
 465 promising adjunctive strategy to optimize therapeutic outcomes in patients.

466 List of abbreviations

Abbreviations	Full Text
AV	Acne vulgaris
ω -3	Ω Omega-3
HC	Healthy controls
AVP	Acne vulgaris patients treated with isotretinoin
AVW	Acne vulgaris patients treated with isotretinoin and ω -3 fatty acids combination
VISIA	VISIA Skin Analysis System Examination
GAGS	Global Acne Grading System
LC-MS	Liquid chromatography-tandem mass spectrometry

⁶ KEGG	¹ Kyoto encyclopedia of genes and genomes
UFAs	Unsaturated fatty acids
LTB4	Leukotriene B4
ApoB	Apolipoprotein B
NAFLD	Nonalcoholic fatty liver disease
GGT	Gamma-glutamyl transpeptidase
HDL-C	High density lipoprotein cholesterol
LDL-C	Low-density lipoprotein cholesterol
VLDL-C	Very low-density cholesterol
ELISA	Enzyme Linked Immunosorbent Assay
FC	Fold Change
VIP	Variable Importance in the Projection
DHA	docosahexaenoic acid
EPA	Eicosapentaenoic Acid
SPMs	specialized pro-resolving mediators
BMI	Body mass index
GAGS	Global Acne Grading System
GLU	Glucose
INS	Insulin
⁶ HOMA-IR	Homeostasis model assessment of insulin resistance
AcCa	Acylcarnitine
BisMePA	Bis-methyl phosphatidic acid
CL	Cardiolipin
DG	Diacylglycerol
Hex2Cer	Hexosylceramide
LdMePE	Lysodi-methylphosphatidylethanolamine
LPC	Lysophosphatidylcholine
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
SQDG	Sulphoquinovosyl Diglyceride
SM	Sphingomyelin
StE	Eicosapentaenoic acid ethylEster
TG	Triglyceride
ZyE	Zymosterol
M	Metabolism
HD	Human Diseases
OS	Organismal Systems
CP	Cellular Processes
EIP	Environmental Information Processing
PLS-DA	¹⁶ Partial Least Squares-Discrimination Analysis
OPLS-DA	Orthogonal Partial Least Squares-Discriminant Analysis
RCT	Randomized Controlled Trial
AA	Arachidonic acid

468 ¹Declarations

469 Ethics approval and consent to participate

470 This research was conducted accord with the Helsinki Guidelines. All patients and
471 healthy volunteers signed the informed consent form, which has obtained approval¹ by
472 the Ethics Committee of the Affiliated Hospital of Southwest Medical University(NO.
473 KY2020115)

474 Consent for publication

475 Not applicable.

476 Availability of data and materials

477 Relevant data are available within the paper and supplement.

478 Competing interests

479 There is no conflict of interest with any financial organization regarding the
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485 Authors' contributions

486 Peiyang Du and Yaxin Huang designed and executed the study and experiments;
487 collected and analyzed the data and drafted the original manuscript. Jie Hu assisted in

488 experiment, data collection and analysis; Yongqiong Deng and Xia Xiong supervised
489 the project, designed the research framework, obtained funding and finalized the
490 study.

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631 **Figure captions**

632 **Figure 1. PLS-DA of untargeted metabolomics data among the samples of**
633 **HC, AVP and AVW groups.** (A) Score plot of cationic metabolites comparing the HC,
634 AVP and AVW groups before intervention. (B) Score plot of anionic metabolites
635 comparing the HC, AVP and AVW groups before intervention. (C) Score plot of
636 cationic metabolites comparing the HC, AVP and AVW groups after intervention. (D)
637 Score plot of anionic metabolites comparing the HC, AVP and AVW groups after
638 intervention. (E) Permutation test plot for the cationic PLS-DA model of HC, AVP
639 and AVW groups before intervention. (F) Permutation test plot for the anionic
640 PLS-DA model of HC, AVP and AVW groups before intervention. (G) Permutation
641 test plot for the cationic PLS-DA model of the HC, AVP and AVW groups after
642 intervention. (H) Permutation test plot for the anionic PLS-DA model of the HC, AVP
643 and AVW groups after intervention. R2Y (cum) and Q2 (cum) are model validation
644 parameters, which represent model interpretability and model predictability,
645 respectively. R2Y and Q2 declined with the decrease of replacement retention, and the
646 regression line showed an upward trend, indicating that there was no overfitting
647 phenomenon in the model. 0, pre-treatment group; 1, post-treatment group.

648 **Figure 2. Volcano plots of lipid metabolites expression after ω -3 fatty acids**
649 **treatment.**(A) Volcano plot of cationic lipid metabolite expression (B) Volcano plot
650 of anionic lipid metabolite expression; Lipid metabolite expression was either
651 down-regulated (blue), up-regulated (red), or unchanged (grey); 0, pre-treatment
652 group; 1, post-treatment group; The horizontal axis denotes the FC value between two

groups in metabolite expression ($\log_2FC$), and the vertical axis denotes the value of the difference ($-\log_{10}(P)$); greater magnitude indicates a more pronounced disparity in expression levels. The size of the point reflecting its VIP value, each data point corresponds to a distinct metabolite. Closer to the left side, right side, or upper part points denotes a more significant difference in expression.

Figure 3. Heatmap of AVW1 and AVW0 in lipid metabolite expression and GAGS score. The color indicates positive correlation (red) or negative correlation (blue). The color intensity is the strength of correlation. The vertical axis on the right shows 30 differentially expressed lipid metabolites, which on the left shows biological functions related to the metabolites. The horizontal axis shows the sample numbers of AVW. The top shows the GAGS score.

Figure 4. KEGG functional enrichment pathways on lipid metabolic before and after ω -3 fatty acids treatment. The abscissa is the enrichment significance P value, and only the pathways with $P < 0.05$ are considered statistically significant and listed in the figure. The ordinate is the name of KEGG pathway. The color of bubbles from blue to red indicates that the P value decreases in turn. The larger the bubble, the more metabolites are enriched in the pathway. 0: pre-treatment group; 1: post-treatment group; M: Metabolism; HD: Human Diseases; OS: Organismal Systems; CP: Cellular Processes; EIP: Environmental Information Processing.

Tables

Table 1. Clinical and laboratory findings of AV patients and healthy controls.

Factors	HC (n=20)	AVP0 (n=20)	AVW0 (n=20)	AVP1 (n=20)	AVW1 (n=20)	P_1	P_2	P_3	P_4
Gender(M/F, n)	18/2	18/2	16/4	8	8	0.71	-	-	-

Factors	HC (n=20)	AVP0 (n=20)	AVW0 (n=20)	AVP1 (n=20)	AVW1 (n=20)	P_1	P_2	P_3	P_4
Age(years)	25.40±2.64	20.15±2.56	21.15±3.57	-	-	0.703	-	-	-
BMI (kg/m ²)	20.09±1.62	20.65±1.73	20.53±2.06	-	-	0.584	-	-	-
Disease duration (months)	-	51.00±24.27	52.10±29.14	-	-	-	-	-	-
Facial GAGS	-	28.60±4.68	27.15±5.21	18.70±4.92	15.05±4.47	-	<0.001	<0.001	0.023
Red area(%)	67.98±17.30	31.63±15.57	26.82±18.14	45.73±19.39	43.87±22.63	<0.001	<0.001	0.006	0.904
Sclererythrin(%)	75.27±8.32	47.72±18.95	52.87±23.67	75.12±18.99	81.40±13.78	0.148	<0.001	<0.001	0.355
①cholesterol(mmol/L)	3.99±0.46	3.86±0.55	3.76±0.67	3.96±0.63	4.10±0.64	0.818	0.277	0.023	0.583
Triglyceride(mmol/L)	0.95±0.43	0.87±0.31	0.73±0.28	1.06±0.35	0.79±0.22	0.067	0.011	0.198	0.01
LDL-C(mmol/L)	2.30±0.36	2.22±0.50	2.11±0.70	2.46±0.62	2.38±0.76	0.728	0.012	0.032	0.738
HDL-C(mmol/L)	1.43±0.27	1.37±0.35	1.49±0.29	1.23±0.26	1.41±0.26	0.04	0.005	0.247	0.026
GLU (mmol/L)	4.59±0.38	4.82±0.24	4.59±0.30	4.63±0.28	4.47±0.22	0.215	0.056	0.14	0.068
INS (pmol/L)	7.85±2.98	6.67±3.32	6.91±3.42	7.33±3.54	9.78±12.19	0.693	0.97	0.156	0.779
HOMA-IR	1.61±0.67	1.44±0.73	1.44±0.77	1.51±0.78	2.00±2.64	0.003	0.007	0.341	0.183

674

675 Note: ①: pre-treatment group; 1: post-treatment group; P_1 , HC vs. AVP0, AVW0; P_2 ,
676 AVP0 vs. AVP1; P_3 , AVW0 vs. AVW1; P_4 , AVP1 vs. AVW1.

677 ① Table 2. Differentially expressed metabolites between AV patients and HC before

678 and after ω-3 fatty acids treatment

Metabolite	HC(n=18)	Acne(n=37)	P_1	AVW0	AVW1	P_2
AcCa(22:0)	6.55±0.2	6.73±0.24	0.003	6.77±0.29	6.23±0.39	0.001
BisMePA(16:2e/20:5)	7.57±0.45	7.23±0.37	0.011	7.12±0.42	7.99±0.3	<0.001
CL(19:0/16:0/20:4/20:4)	6.23±0.37	6.09±0.36	0.315	6.01±0.25	6.45±0.41	0.003
DG(10:0/1④)	4.87±0.33	6.49±0.52	<0.001	6.47±0.5	5.68±0.92	0.016
DG(18:1/22:6)	6.79±0.37	4.78±2.41	<0.001	5.04±2.28	7.07±0.39	<0.001
DG(20:5/18:2)	6.98±0.43	6.34±0.45	<0.001	6.32±0.42	7.22±0.28	<0.001
DG(9:0/11:2)	5.5±0.42	7.45±0.85	<0.001	7.52±0.94	6.26±1.34	0.003
Hex1Cer(t18:0/20:5)	6.2±0.27	6.15±0.36	0.72	6.08±0.31	6.53±0.3	0.002
LdMePE(20:5)	6±0.44	6.09±0.25	0.451	6.02±0.22	6.57±0.34	<0.001
LPC(1⑬)	7.51±0.31	7.65±0.23	0.119	7.63±0.21	8.21±0.33	<0.001
PC(16:0/8:0)	4.55±1.54	5.23±0.77	0.014	5.43±0.53	4.75±0.89	0.03
PC(16:1/22:6)	6.95±0.41	6.61±0.44	0.005	6.58±0.47	7.07±0.23	0.001
PC(16:1e/20:5)	6.43±0.28	6.39±0.24	0.886	6.33±0.18	6.77±0.32	0.002
PC(18:3/2③)	7.12±0.25	6.99±0.32	0.041	7.01±0.34	7.54±0.42	0.005
PE(14:1e/22:6)	5.7±0.36	5.3±0.47	0.003	5.26±0.44	5.79±0.37	0.002
PE(16:1e/20:5)	7.23±0.38	7.01±0.28	0.031	6.97±0.26	7.51±0.33	<0.001
SM(d20:1/23:4)	7.61±0.32	8.27±0.37	<0.001	8.33±0.36	7.57±0.56	0.001
SQDG(18:2/22:4)	5.36±0.49	5.55±0.54	0.167	5.53±0.62	5.15±0.49	0.033
St②(20:5)	6.93±0.21	6.46±0.35	<0.001	6.44±0.33	7.18±0.22	<0.001
TG(16:0/22:6/22:6)	7.24±0.55	7.05±0.44	0.11	7.11±0.38	7.78±0.44	0.001
TG(17:0/1④/22:6)	7.09±0.48	6.56±0.52	0.001	6.5±0.51	7.36±0.2	<0.001
TG(18:0/20:4/22:6)	7.06±0.74	7.1±0.41	1	7.09±0.43	7.62±0.34	0.006
TG(18:1/22:6/22:6)	7.1±0.47	6.88±0.46	0.141	6.88±0.53	7.77±0.41	0.001
TG(18:2/20:4/22:6)	8.01±0.39	7.76±0.23	0.009	7.72±0.24	8.3±0.36	<0.001
TG(18:2/22:6/22:6)	7±0.5	6.78±0.4	0.167	6.84±0.39	7.66±0.47	<0.001
TG(20:5/18:2/22:6)	7.08±0.53	6.93±0.39	0.578	6.95±0.38	7.72±0.46	0.001
TG(22:5/18:2/20:4)	7.29±0.36	6.91±0.28	<0.001	6.9±0.32	7.41±0.34	0.001
TG(22:5/18:2/22:6)	6.73±0.42	6.78±0.4	0.53	6.77±0.4	7.3±0.3	0.001
TG(24:2/10:1/12:1)	5.94±0.45	6.24±0.58	0.026	6.38±0.59	5.93±0.38	0.003
ZyE(22:5)	6.68±0.46	6.61±0.31	0.462	6.66±0.35	7.19±0.26	<0.001

679 Note: ①: pre-treatment group; 1: post-treatment group; P_1 , HC vs. AV; P_2 , AVW₀

680 vs. AVW₁. ① AcCa, acylcarnitine; BisMePA, bis-methyl phosphatidic acid; CL,

681 cardiolipin; DG, diacylglycerol; Hex2Cer, hexosylceramide; LdMePE,

682 Lysodi-methylphosphatidylethanolamine ;LPC, lysophosphatidylcholine; PC,

683 phosphatidylcholine; PE, phosphatidylethanolamine; SQDG, Sulphoquinovosyl
684 Diglyceride; SM, sphingomyelin; StE, eicosapentaenoic acid ethyle Ester; ZyE,
685 Zymosterol