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Systemic responses to oral hyaluronic acid: A clinical randomized placebo-controlled study of plasma immune and metabolomic responses

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Abstract

Oral hyaluronan (HA) supplementation has shown clinical benefits, but its systemic effects remain insufficiently characterized. We focused on the systemic effects of oral HA (1.8 MDa) at 60 mg/day (SH60), 120 mg/day (SH120), or placebo for 12 weeks in 150 healthy donors. Within this randomized, double-blind, placebo-controlled study, plasma samples were analyzed to assess changes in immune and metabolic markers, as well as alterations in lipid metabolism, using high-quality, untargeted omics approaches with high information depth. The SH120 group significantly reduced pro-inflammatory markers, including CRP, IL-1b, and precursor of inflammatory mediators such as arachidonic acid, compared with placebo. Together with selective modulation in metabolic biomarkers, including fetuin-A, adipsin, mannopyranosyl tryptophan, 1,16-hexadecanedioic acid, and 3-hydroxydecanoic acid, supports the hypothesis that oral HA may promote an immune homeostasis and a “metabolically healthy” profile, exerting protective effects even in otherwise healthy individuals. Correlations between plasma markers and previously reported skin outcomes were generally weak, suggesting that modulation of systemic biomarkers can explain only partially the cutaneous effects of supplementation. Overall, oral HA was associated with selective changes in plasma biomarkers in healthy adults, which should be considered when evaluating the mechanisms underlying its clinical effects. Trial registration: NCT07065110.

1. INTRODUCTION

Hyaluronan (HA) is an endogenous glycosaminoglycan exhibiting intrinsic biocompatibility, biodegradability, and non-immunogenic properties. It plays a pivotal role in a wide range of biological and pathophysiological processes. HA can be administered via multiple routes, including oral supplementation. Several clinical studies have demonstrated that orally administered HA exerts beneficial effects in the management of joint disorders [1, 2], inflammatory bowel diseases [3, 4], and improves skin properties, including enhanced hydration [5, 6]. Despite this evidence, the precise mechanism of action of oral HA remains insufficiently understood. To date, most clinical studies investigating oral HA in humans were conducted in populations with underlying pathologies, such as joint disorders or inflammatory disease, mainly assessing clinical parameters such as joint pain or skin hydration [7–10] without assessing systemic parameters. Several animal studies suggested that the beneficial effects of oral HA on skin or joints are connected with changes in systemic biomarkers connected with inflammation and metabolic health [11–13]. Some reports monitored several systemic circulating biomarkers in patients with obesity or joint inflammation [14, 15]. Importantly, high–molecular weight HA (HMW-HA) is degraded into smaller fragments within the gastrointestinal tract and exhibits limited systemic bioavailability [13, 16]. Thus, the HA effects on skin [5, 6] and osteoarthritis [15, 17] are likely mediated by its systemic effect rather than direct deposition in the distal organs. However, clinical evidence describing systemic effects of oral HA using exploratory omics methods is completely lacking, and overall systemic evaluation of orally administered HA remains scarce. The present study addresses this gap by providing the extended evaluation of systemic responses to oral HA supplementation in healthy humans. Within the framework of this study, skin-related outcomes have already been reported [5], demonstrating that oral HA increases skin hydration, reduces transepidermal water loss (TEWL),

wrinkle depth, and sebum levels, and enhances skin elasticity. Herein we investigated whether oral administration of HA induces systemic changes in healthy adults beyond the previously reported skin-related outcomes. We hypothesized that orally administered HA, at clinically relevant doses, would be associated with specific changes in inflammatory and metabolic plasma markers, consistent with a systemic response to supplementation, and that these changes might relate to the previously observed skin effects. We therefore analyzed paired plasma samples collected before and after 12 weeks of supplementation using multiplex immunoassays, untargeted metabolomics, untargeted lipidomics, and targeted oxylipin analysis. This integrative approach aims to advance understanding of the mechanisms underlying oral HA's effects in target tissues and to elucidate its potential role within the gut–skin axis.

2. MATERIALS AND METHODS

2.1 Study design, randomization, and ethical considerations

This was a single-center, double-blind, randomized, placebo-controlled, parallel-group clinical trial conducted in accordance with the SPIRIT 2013 guidelines [18]. HA (Nutrihyl®, food-grade) used in this study was of microbial origin (*Streptococcus equi subs. zooepidemicus*) and manufactured by Contipro a.s. (Czech Rep.) and had a molecular weight (M_w) of 1.8 MDa. Xanthan gum, a widely used food additive, was chosen as a placebo to mimic the viscoelastic properties of HA solutions. Xanthan gum is a widely used food additive found in various consumer products in Europe20 suggesting that the gut microbiome of most individuals is probably adapted to its presence, discussed in more details in [5]. Eligible participants were recruited between June and August 2024. The intervention and clinical assessments were performed from September to December 2024.

Participants obtained altogether 3 flasks containing 500 ml of 60 mg day^{-1} of HA (SH60), 120 mg day^{-1} of HA (SH120), or a placebo solution. 50 participants per group (150 total) took 15 ml of the assigned solution once daily in the morning for 3 months. (Fig. 1). The three solutions were indistinguishable in appearance, taste, and smell and packaged in identical opaque flasks. Participant adherence was assessed by self-reported missed doses, recorded biweekly in the questionnaire, and by weighing returned bottles containing residual solutions at each visit. Average daily intake (ml) was calculated from the weight difference of supplement bottles at each study visit (assuming 1.0 g ml^{-1} density).

The study was intentionally designed to reflect a representative healthy population, encompassing both female and male participants across multiple age categories. Its primary outcome was to investigate the effect of HA on skin hydration, which was reported in [5]. The secondary outcome, and the focus of this article, were exploratory omics analyses of blood samples. Inclusion criteria: age 18–60 years, Caucasian ethnicity, Fitzpatrick skin phototype I–III, no acute/chronic skin or gastrointestinal disorders, and willingness to maintain usual skincare, avoid SH-containing supplements, excessive UV exposure, and prolonged stays in different climates. Exclusion criteria: pregnancy/lactation, allergy to study

products, antibiotic or SH supplement use within the previous 3 months, and diabetes mellitus. The sample size calculation was based on an expected between-group difference of 5 AU (≈ 8.6%) in cheek hydration after 3 autumn months, assuming a conservative standard deviation of 15%. To detect this effect with 80% power and a two-sided alpha of 0.05 (two-sample t-test), 49 participants per group were required. Participants were stratified by sex (male/female) and age group (18–39 and 40–60 years), and randomly assigned to placebo, SH60 and SH120 groups using the RAND() function in Microsoft Excel based on anonymized, stratified participant codes. The allocation key was securely stored and inaccessible until study completion. Unblinding was allowed only in the event of a serious adverse event. Harms were assessed systematically using biweekly safety questionnaires and spontaneous participant reporting. Interim analyses were not performed. The participant flow diagram is presented in Fig. 2. The details of this randomized placebo-controlled study are described in detail in [5]. Baseline characteristics of participants included in the primary outcome analysis are summarized in Table 1.

The study was registered in the EFSA (The European Food Safety Authority) trial registry (ID: EFSA-2024–00027979) prior to participant enrolment (prospectively filed on 06 Jun 2024). The trial was then registered retrospectively at ClinicalTrials.gov (ID: NCT07065110) in accordance with journal transparency requirements (retrospectively registered on 15 Jul 2025). The study was conducted at the facilities of Contipro a.s. (Dolní Dobrouč, Czech Republic). [5]

The study was conducted in accordance with the Declaration of Helsinki (2013 revision), and the study protocol was reviewed and approved by the Contipro Research Ethics Committee prior to study initiation (ID: AICE24_ II, approved on 13th June 2024). Written informed consent was obtained from all participants before the study began. Data confidentiality was ensured through anonymization and secure data handling in compliance with the General Data Protection Regulation (EU 2016/679). [5]

Table 1
Baseline characteristics of participants included in analyses of plasma samples.

number	**placebo**	**SH60**	**SH120**
	50	**48**	**50**
female/male	32/18	31/16	32/18
age, mean ± SD	43.4 ± 10.8	44.3 ± 8.8	43.8 ± 9.2
age, range	18–60	22–59	24–60

2.2 Quantification of protein targets in plasma

Blood from healthy humans (4 ml from the medium cubital vein) was collected in EDTA tubes and centrifuged (1500g, 10 minutes, 4°C). Plasma was collected and stored at -80°C. Plasma from the placebo and SH120 groups was thawed and kept on ice. The levels of EGF, FGF-2, IFN-γ, IL-1β, IL-1RA, IL-10, IL-17A (in multiplex Milliplex Human cytokine/chemokine/growth factor panel A magnetic bead panel, cat. number HCYTA-60K, Merck Life Science), C-peptide, GLP-1, glucagon, IL-6, leptin, MCP-1, TNF-

α (in multiplex Milliplex Metabolic hormone panel V3, cat. number HMH3-34K, Merck Life Science), and CRP, fetuin-A, adipsin (in multiplex Milliplex Human cardiovascular disease magnetic bead panel 3, cat. number HCVD3MAG-67K, Merck Life Science) were measured according to the manufacturer's instructions. The signal was acquired with the Magpix instrument (Luminex, Austin). Plasma samples were diluted 1:1000 in assay buffer for the multiplex HCVD3MAG-67K kit. Plasma samples were not diluted for the other two multiplex kits. The values are related to the standard curves and expressed in pg ml^{-1} or ng ml^{-1}. The detection limits were 3 pg ml^{-1} for EGF, 26 pg ml^{-1} for FGF-2, 1.3 pg ml^{-1} for IFN-γ, 1.6 pg ml^{-1} for IL-1β, 1.6 pg ml^{-1} for IL-1RA, 2.6 pg ml^{-1} for IL-10, 1.3 pg ml^{-1} for IL-17A, 27 pg ml^{-1} for C-peptide, 3.4 pg ml^{-1} for GLP-1, 6.9 pg ml^{-1} for glucagon, 2.7 pg ml^{-1} for IL-6, 137 pg ml^{-1} for leptin, 6.9 pg ml^{-1} for MCP-1, 1.0 pg ml^{-1} for TNF-α, 0.012 ng ml^{-1} for CRP, 0.244 ng ml^{-1} for fetuin-A, 0.098 pg ml^{-1} for adipsin. Twenty-one values for fetuin-A and adipsin were excluded due to an analytical error.

The levels of adiponectin (cat. number EZHADP-61K, Merck Life Science), adropin (cat. number E-EL-H5307, Elabscience), and IL-8 (cat. number 88-8086-88, Invitrogen) were measured according to the ELISA manufacturer's instructions. The absorbance was read by an EnSight Multimode Reader (PerkinElmer, Waltham, MA, USA). Plasma samples were diluted 1:500 in assay buffer for the adiponectin ELISA assay and 1:20 for the adropin ELISA assay. The values are related to the standard curves and expressed in pg ml^{-1} or ng ml^{-1}. The detection limits were 1.5 ng ml^{-1} for adiponectin, 12.5 pg ml^{-1} for adropin, and 4 pg ml^{-1} for IL-8.

2.3 Metabolomic analysis

Low-molecular-weight metabolites from 100 µl of plasma were extracted with 400 µl of chilled (-80°C) methanol. Methanolic extracts were dried under a stream of nitrogen and dissolved in 100 µl of 0.1% formic acid. A Vanquish Horizon UHPLC system coupled with an Exploris 240 (Thermo Fisher Scientific) mass spectrometer equipped with an electrospray ionization source was used. RPLC was performed according to [19] on an Acquity C18 column UPLC HSS T3 (2.1×100 mm, 1.8 µm) (Waters) with a gradient elution of 0.2% formic acid in water (v/v) and 0.1% formic acid in acetonitrile (v/v). Metabolites were detected in negative ion mode, utilizing an ion transfer tube temperature of 350°C, a sheath gas pressure of 60 psi, an auxiliary gas flow of 9, and a spray voltage of 3.4 kV. MS data (*m/z* 60–900) were collected using a dynamic exclusion list and a targeted inclusion list updated in the AcquireX data acquisition mode to achieve optimal metabolite identification in Compound Discoverer 3.4 software (Thermo Fisher Scientific, Waltham, USA). [12]

2.4 Lipidomic analysis

The plasma samples were thawed on ice. 80 µl of solution was transferred to a microtube, followed by the addition of 300 µl of cooled (-20°C) isopropanol containing a mixture of internal standards. The microtubes were vortexed (2000 rpm, 2 minutes) and stored in the freezer (-20°C) overnight for complete protein precipitation. The solutions were centrifuged, transferred to vials, and analyzed by LC-MS. A

pooled sample solution was prepared by mixing aliquots of each sample, followed by the same procedure as standard samples.

Samples were analyzed using an in-house untargeted lipidomic method using RP-UHPLC-MS using DDA in positive and negative modes on Orbitrap Exploris 240. The original ACQUITY BEH C18 1.7 µm, 2.1x50 mm was used for reverse-phase chromatography. Positive and negative modes were run separately, using different mobile-phase modifiers. Mobile phase A was Acetonitrile:water (3:2) and B was Isopropanol:Acetonitrile (9:1). For positive mode, the mobile phases were modified with 10 mM NH_4COOH and 10 mM CH_3COONH_4 for the negative one. Gradient was as follows 0–1 min 15–30% B, 1-1.3 min 30–48% B, 1.3–5.5 min 48–82% B, 5.5–5.8 min 82–99% B, 5.8-6 min 99% B, 6-6.1 min 99 – 15% B, 6.1-7 min 15% B. Flow rate 0.6 ml/min. The column and autosampler temperatures were 65°C and 10°C, respectively. The injection volume was 3 µl. MS parameters were as follows: Positive capillary voltage 3500 V, Negative capillary voltage 2000 V, Sheath gas 35 arb, Auxiliary gas 10 arb, Sweep gas 0 arb, Ion Transfer Tube temperature 300°C, Vaporizer temperature 350°C, RF Lens 75%, Full MS resolution 45000, Full MS range 200–1700 m/z, ddMS resolution 11250, Window width 0.7 Da. Every sample was injected once. Blank and pooled samples were injected after every tenth sample injection. The order of injected samples was randomized.

The data were processed using Compound Discoverer 3.3, and peaks were manually inspected. Wrongly integrated peaks were corrected using Tracefinder. The unique peak areas were processed in Excel to perform calculations.

2.5 Oxylipin analysis

Samples for oxylipin analysis were prepared using an economic approach by evaporating 200 µl of lipid extract in a rotary vacuum evaporator. The dried extract was redissolved in 100 µl Water:Acetonitrile:Acetic acid (59:39:2) using a vortex (10 min, 2000 rpm) and transferred to vials. Samples were analyzed using RP-UHPLC coupled to TSQ Altis Plus™ Triple Quadrupole mass spectrometer in negative SRM mode. ACQUITY HSS T3 1.8 µm, 2.1x100 mm, and water and acetonitrile, both modified with 0.02% Acetic acid were used for separation. The gradient was as follows: 0-0.5 min – 30% B, 0.5-1 min 40% B, 1-2.5 min 40% B, 2.5–4.5 min 40–70% B, 4.5–6.5 min 70% B, 6.5-9 min 95% B, 9–12 min 95% B, 12-12.1 min 95 – 30% B, 12.1–14.1 min 30% B. Flow rate 0.3 ml/min. The column and autosampler temperatures were 40°C and 4°C, respectively. Injection volume was 10 µl. MS conditions Negative capillary voltage 2500 V, Sheath gas 45°C, Auxiliary gas 15 arb, Sweep gas 1 arb, Ion Transfer Tube temperature 350°C, Vaporizer temperature 350°C, Q1 resolution 1.2 Da, Q2 Resolution 1.2 Da.

2.6 Correlation between cosmetics parameters and plasma analytes

For the correlation analysis, cosmetic parameters M3/M0 ratios altered by HA treatment [5] were correlated with M3/M0 ratios of selected plasma markers using Spearman's correlation in RStudio. The

heatmaps were used for visualization. Placebo and SH120 were correlated and visualized separately to determine whether specific markers correlate similarly after SH120 treatment as they do with placebo.

2.7 Statistical analysis

The data were presented as the median, 25th and 75th percentiles, min, and max. To assess treatment-induced changes, the results from individual donors were calculated as the ratio of month 3 to month 0 (M3/M0). Data distribution normality was evaluated using the Kolmogorov-Smirnov test. Although the majority of the datasets met the assumption of a normal distribution, non-parametric statistical methods were systematically applied to maintain a uniform analytical approach across all evaluated parameters. For all statistical analyses, a *p*-value of less than 0.05 was considered to indicate statistical significance.

For the multiplex Magpix M3/M0 data, the Mann-Whitney *U* test was used to evaluate differences between the placebo and SH120 groups. For the high-dimensional omics datasets (metabolomics and lipidomics), the Kruskal-Wallis test followed by Dunn's post-hoc test with Benjamini-Hochberg false discovery rate (FDR) correction was used to compare multiple experimental groups (placebo vs. SH60, placebo vs. SH120).

Effect magnitudes for univariate comparisons were quantified using Hedges' *g*. Dose-dependent relationships and multi-variable treatment effects across the omics blocks were further examined using sparse Partial Least Squares (sPLS) regression, focusing on the separation of treatment groups along the top latent components. To evaluate the associations between clinical/cosmetic outcomes and systemic plasma biomarkers, Spearman's rank-order correlation analysis was performed using the M3/M0 ratios, and the resulting correlation matrices were visualized using heatmaps with hierarchical clustering.

In lipidomics, individual lipid concentrations were quantified relative to the known concentration of the corresponding internal standard. Normalized concentration values were calculated using probabilistic quotient normalization on the structural lipids. [20] Afterward, the ratio M3/M0 was calculated. The fold change was calculated as the M3/M0 ratio divided by the average of this ratio across all lipids in the dataset.

Principal Component Analysis (PCA) was performed as an unsupervised exploratory step to visualize global metabolic profiles and detect potential clustering. To quantify the overall metabolic shift for each subject over the course of the study, the Euclidean distance was calculated between their baseline (M0) and post-treatment (M3) coordinates within the scores space of the first two principal components (PC1/PC2). Statistical computing and visualization were conducted using the Metaboanalyst [21] and R software (v4.5.2). Specifically, effect size computations were carried out using the *effsize* (v0.8.1) package, and multi-block sPLS analyses were performed utilizing the *mixOmics* (v6.32.0) package.

2.8 Data availability

Datasets related to this article can be found at https://doi.org/10.5281/zenodo.20930953, an open-source online data repository hosted at Zenodo.

3. RESULTS

3.1 Oral HA intake modulates plasma proteins linked to immune and metabolic regulation

Plasma samples from the placebo and SH120 groups were analysed by multiplex and ELISA assays targeting immune and metabolic markers. The most pronounced effects were observed in the group supplemented with 120 mg day^{-1} of HA (SH120). Proinflammatory markers interleukin-1β (IL-1β) and C-reactive protein (CRP) were significantly decreased in the SH120 group after 3 months of supplementation compared to the placebo group (Fig. 3). Interestingly, statistically significant differences were observed despite the fact that these were healthy volunteers with physiologically very low levels of these mediators. Other proinflammatory analytes, IL-17A, interferon-gamma (IFN-γ), and tumor necrosis factor-α (TNF-α), revealed decreasing trends; however, these changes did not reach statistical significance. SH120 significantly reduced the levels of IL-10, an anti-inflammatory cytokine, and interleukin-1 receptor antagonist (IL-1RA), which is an IL-1β receptor antagonist. The observed reduction in IL-10 and IL-1RA may reflect a secondary response to decreased levels of the pro-inflammatory markers IL-1β and CRP, indicating a reduced requirement for anti-inflammatory regulation. The levels of growth factors epidermal growth factor (EGF) and fibroblasts growth factor 2 (FGF2) were unchanged (Fig. 3). The proinflammatory cytokine IL-8 was under the detection limit (data not shown). Interestingly, a minor variance in SH120 compared to placebo was observed for most analytes; thus, HA-supplemented samples displayed lower dispersion (Fig. 3, 4). Additionally, CRP and INF-γ reached medium effect sizes, which supported the biological relevance of the observed changes (Figure S2). Overall, these results point to a positive effect of oral HA and support for maintaining immune homeostasis.

To focus on the metabolic changes associated with HA supplementation, based on the literature and our previous studies, we identified markers associated primarily with lipid and carbohydrate metabolism. Fetuin-A and adipsin, which are closely associated with metabolic syndrome, obesity, inflammation, and insulin resistance, were significantly reduced after HA supplementation (Fig. 4). Glucagon-like peptide 1 (GLP-1) (natural gut hormone), glucagon (insulin antagonist), and adiponectin (adipokine) remained unchanged (Fig. 4). Likewise, C-peptide, IL-6, leptin, Monocyte Chemoattractant protein-1 (MCP-1), and adropin showed no statistically significant alterations (Figure S3).

3.2 Lipidomic and metabolomic analysis reveals selective plasma metabolite modulation without broad lipidome or metabolome remodelling by oral HA

To explore potential systemic mechanisms behind the clinically observed skin benefits, untargeted lipidomic and metabolomic profiling of plasma was conducted to identify metabolic alterations

associated with oral HA supplementation. The lipidomic analysis identified 532 unique lipids from 26 lipid classes. The principal component analysis (PCA) and partial least square discriminant analysis did not separate the groups according to the difference between month M3/M0 (plots not shown), and lipidomic data did not reach statistically significant changes that would indicate a consistent trend at the SH60 and SH120 doses (Fig. 5A). Figure 5A also displayed the individual samples together with the pooled quality control (QC) to visualize data quality. The main driver of lipidomics data was the individual subject (Figure S4). According to our lipidomic analysis, the average composition of the plasma lipidome was mainly composed of cholesteryl esters, cholesterol, triacylglycerols, and phosphatidylcholines (Fig. 5B). Oral HA did not adversely affect systemic plasma lipid profile in healthy donors.

Untargeted metabolomic analysis of plasma identified 137 unique metabolites and revealed that HA supplementation exerted only minor overall changes in comparison with placebo, as illustrated by PCA (Fig. 5C). Figure 5C also illustrates the individual samples and the pooled QC to demonstrate data quality. As observed in the lipidomics analysis, the data are driven predominantly by inter-individual variability (Figure S5). However, statistically significant metabolites were identified by the Mann-Whitney test for placebo vs SH120 and confirmed by library matching (Fig. 5D).

Metabolically and biologically related markers that can be associated with the beneficial effects of HA are shown in detail in Fig. 4E. Oral HA influences specific metabolites in the healthy human plasma. Notably, HA supplementation significantly decreased mannopyranosyl tryptophan, 1,16-hexadecanedioic acid, 3-hydroxydecanoic acid, and 2-furoylglycine, the reduction of which is considered a beneficial effect for metabolic health. The reduced oxidative damage to polyunsaturated fatty acids due to HA supplementation was also supported by a slight downward trend in malondialdehyde with increasing HA doses; however, the statistical significance threshold ($p < 0.05$) was not exceeded (Figure S6C). Additionally, exogenous metabolite 2,6-dihydroxybenzoic acid showed a trend toward increased levels following HA supplementation ($p = 0.06$). The oxylipin analysis identified only 4 oxylipins in plasma (12,13-diHOME, 13-HODE, 9-HODE, 5-HETE) and 3 free fatty acids (arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid). Changes in oxylipins and fatty acids, except for arachidonic acid, did not reach statistical significance, as expected given that the subjects were healthy individuals. (Figure S6A, B). However, plasma levels of arachidonic acid were significantly reduced, corroborating the observed decreases in pro-inflammatory markers (e.g. IL-1β, CRP) (Fig. 5E). Additionally, the effect size analysis showed that the differences between the placebo and SH120 groups were not only statistically significant but also reached medium to large effect sizes for mannopyranosyl tryptophan, 2-furoylglycine, and arachidonic acid, underscoring the clinical and biological relevance of these metabolic changes (Figure S2).

3.3 Dose-dependent effects of oral HA on lipid and metabolite levels

The objective of dose-response analysis was not to maximize statistical significance, but rather to evaluate the consistency and directionality of changes across dose levels. Dose-response analysis was

therefore restricted to metabolomic and lipidomic datasets. Multiplex magpix parameters (only placebo and SH120 were analysed) could not be incorporated into the analysis, representing a limitation of the current multivariate model. The analysis revealed that a substantial proportion of the selected parameters exhibited monotonic dose-dependent responses to HA administration. This pattern is consistent with a biologically meaningful dose-responsive effect rather than random intergroup variability. The resulting output does not represent a set of independent biomarkers but rather a linear combination of variables capturing a shared underlying biological effect (Figure S7). The final Sparse Partial Least Squares (sPLS) signature comprised seven selected variables that demonstrated the strongest statistical contribution, including their respective directional associations (+/−) and relative weights (Fig. 6). The composite score derived from the sPLS signature exhibited a clear dose-dependent shift across the study groups. Whereas the placebo group was characterized predominantly by negative score values, the HA-treated groups showed a progressive increase, with the highest scores observed in the SH120 group. Notably, this dose-responsive pattern was driven not only by global multivariate structure, but also predominantly by analytes such as mannopyranosyl tryptophan, arachidonic acid, and 2-furoylglycine (Fig. 6).

3.4 Correlation of oral HA–associated skin improvements with changes in plasma biomarkers

To determine whether the previously observed beneficial effects of oral HA on skin vitality - namely reduced wrinkle formation, decreased TEWL, and enhanced skin hydration and elasticity (described in detail by [5] were accompanied by systemic biochemical alterations in the plasma, correlation analyses were performed between skin-related outcomes and the above-mentioned plasma analytes. To assess the magnitude and robustness of the overall effects associated with HA supplementation, a comprehensive one-dimensional statistical analysis was performed as an initial screening step to identify parameters potentially most sensitive to HA supplementation across all measured variables, including cosmetic, multiplex, metabolomic, and lipidomic datasets, together with false discovery rate (FDR) correction for multiple analyses. Collectively, the results demonstrated that HA supplementation exerted the most pronounced effects on adipsin, fetuin-A, wrinkle parameters, and TEWL (Figure S8). Correlation analyses based on M3/M0 ratios revealed overall weak associations between cosmetic parameters and circulating biomarkers in the SH120 group (Fig. 7) and the placebo group (Figure S9). Only increased skin hydration exhibited a moderate positive correlation with reduced CRP plasma concentrations (S rho = 0.5). All remaining associations were weak, with correlation coefficients of ± 0.3 or lower. These included, for example, improved skin hydration, which was positively associated with lower levels of IL-17A; while negative correlating with reductions in 3-hydroxydecanoic acid and IL-1RA. As well as decreased TEWL, it was inversely associated with a reduction in IL-17A. Also, increased elasticity correlated positively with higher 2,6-dihydroxybenzoic acid (Fig. 7). Nevertheless, although biologically relevant trends were identified for these analytes, the correlation analysis fails to implicate a consistent mediating mechanism.

DISCUSSION

This randomized, placebo-controlled clinical trial provides evidence that oral supplementation with HMW HA (1.8 MDa) at a daily dose of 120 mg improves immune homeostasis and modulates several metabolic biomarkers in the plasma of healthy humans. This was accompanied by beneficial effects on skin parameters, specifically showing wrinkle reduction, decreased TEWL, and enhanced hydration and elasticity in healthy Caucasian subjects [5]. Although the precise molecular mechanisms underlying these effects remain incompletely understood, our findings support a multifactorial mode of action involving immune modulation, regulation of lipid and carbohydrate metabolism. To date, the effects of oral HA on circulating cytokines and metabolic markers have not been systematically investigated in healthy human subjects or animal models, rendering the present data both novel and unique.

A central finding of this study is the significant reduction in key pro-inflammatory markers - IL-1β and CRP in the SH120 group compared with placebo. Importantly, these observed reductions in immune analytes are accompanied by significantly decreased plasma arachidonic acid levels, further supporting the immune-regulating role of oral HA. HA supplementation was associated with increased levels of 2,6-dihydroxybenzoic acid, a metabolite previously linked to favourable cardiometabolic outcomes and modulation of NF-κB signaling pathways [22, 23]. Collectively, these findings indicate that oral HA may promote immune homeostasis even in individuals without overt inflammatory disease.

IL-1β is a pivotal cytokine in innate immunity and plays a critical role in the pathogenesis of chronic inflammatory diseases. To our knowledge, this is the first report demonstrating reduced circulating IL-1β concentrations following oral HA supplementation in healthy individuals. Previous evidence has largely been restricted to disease settings. For example, intra-articular HA administration reduced IL-1β levels in synovial fluid from patients with osteoarthritis [24]), while oral HA decreased the expression of IL-1β, TNF-α, and IL-6 in experimental osteoarthritis models [17, 25]. Our findings, therefore, suggest that the immunomodulatory properties of HA may extend beyond pathological conditions and can be detected under physiological conditions.

The reduction in CRP further supports this interpretation. CRP is a clinically established marker of systemic inflammation and a recognized predictor of cardiometabolic risk. In addition, CRP reduction is a therapeutic strategy in chronic inflammatory and metabolic disorders [26]. Interestingly, increased concentrations of 2,6-dihydroxybenzoic acid have previously been associated with lower CRP levels and improved cardiometabolic profiles [23], consistent with the direction of changes observed in the present study. However, previous clinical studies investigating oral HA in healthy volunteers or individuals with knee discomfort reported no significant changes in circulating CRP concentrations [15, 27].

The observed reduction in arachidonic acid provides additional mechanistic support for the immunomodulation effects of oral HA. Arachidonic acid serves as a major precursor of prostaglandins, leukotrienes, and other lipid mediators that regulate immune responses. Previous studies have shown that HA suppresses arachidonic acid metabolism by reducing the activity or expression of cyclooxygenase and lipoxygenase pathways. In animal models of osteoarthritis, oral HA decreased cyclooxygenase-2 expression and reduced prostaglandin E2 (PGE2) production, while simultaneously

lowering leukotriene B4 levels by suppressing 5-lipoxygenase activity [25]. Similar effects have been reported in macrophages and synovial cells, where HA inhibits PGE2 production through CD44-mediated signaling pathways. [17, 28] Because the present study was conducted in healthy individuals, most downstream eicosanoids were present at very low concentrations and did not reach statistical significance. Nevertheless, our recent murine study of UV-induced photoaging similarly demonstrated reduced plasma and skin levels of PGE2 and PGD2 following oral HA supplementation [12], further supporting modulation of inflammatory lipid signaling as a plausible mechanism of action.

The concomitant reductions in IL-10 and IL-1RA may initially appear counterintuitive, given their established anti-inflammatory functions. However, both mediators are frequently induced as compensatory responses to ongoing inflammatory signalling. Their reduction in the SH120 group may therefore reflect a decreased requirement for endogenous anti-inflammatory counter-regulation following attenuation of upstream inflammatory pathways, particularly those involving IL-1β [29]. This interpretation is consistent with previous studies reporting unchanged IL-10 concentrations in plasma or synovial fluid despite clinical benefits of oral HA supplementation, such as alleviating knee discomfort [10, 15].

Beyond its effects on immune markers, oral HA supplementation also induced selective changes in circulating metabolic biomarkers. Notably, the most pronounced metabolic effects were observed for fetuin-A and adipsin, both of which were significantly reduced following three months of supplementation. These changes occurred in the absence of major alterations in adiponectin, GLP-1, glucagon, adropin, leptin, and C-peptide, suggesting that oral HA does not broadly perturb systemic metabolism but rather affects specific pathways associated with metabolic regulation.

Fetuin-A is a liver-derived hepatokine that has emerged as a clinically relevant marker of metabolic dysfunction [30]. Reduction in fetuin-A is widely interpreted as a marker of improved insulin sensitivity and metabolic health. The interpretation of decreased adipsin concentrations is not straightforward, as circulating adipsin levels are elevated during rheumatoid arthritis [34], metabolic syndrome, and the early stages of type 2 diabetes [35], while its decline in later disease stages indicates progressive β-cell dysfunction and failure [36]. The simultaneous reduction of both fetuin-A and adipsin is consistent with several of our previous experimental studies. In healthy mice, oral HA supplementation altered hepatic energy metabolism and reduced glycogen breakdown, suggesting improved metabolic efficiency [13]. Similarly, Huang et al. demonstrated increased hepatic glycogen content following oral HA administration (50 mg/kg/day, 1ž5 days) in healthy animals [11], while studies in high-fat models reported improvements in insulin sensitivity, enhanced lipolysis, and reduced fat accumulation [37]. Conversely, other previous studies have demonstrated that HA supplementation did not affect metabolic panel analytes, liver enzymes, or blood biochemical parameters, including glucose concentrations, in volunteers experiencing chronic pain [7] or knee discomfort [15].

The metabolomic analyses further support the concept of systemic immune–metabolic modulation induced by oral HA. Although oral HA did not induce large-scale remodelling of the plasma metabolome

or lipidome, several metabolites associated with metabolic health were significantly altered. In particular, reduced levels of mannopyranosyl tryptophan, 1,16-hexadecanedioic acid, 3-hydroxydecanoic acid, and 2-furoylglycine. The observed decrease in mannopyranosyl tryptophan concentrations, accompanied by a strong dose-dependent effect, may indicate a potentially beneficial metabolic effect of oral HA, as elevated levels have been previously associated with chronic low-level inflammation [38] and type 2 diabetes [39]. Similarly, 3-hydroxydecanoic acid, significantly decreased in our study, is primarily recognized in clinical research as an inflammatory biomarker associated with metabolic syndrome and type 2 diabetes [40]. Similarly, reduced concentrations of 1,16-hexadecanedioic acid may indicate a beneficial metabolic effect, as lower levels of this metabolite have been linked to a more favourable cardiometabolic profile and reduced blood pressure [41]. Although individual metabolomic changes were modest, the combined molecular signature demonstrated a clear progression from placebo to SH60 and SH120 groups, indicating that oral HA induces coordinated biological responses across multiple metabolic pathways.

To assess whether systemic changes relate to the reported skin effects [5], correlation analyses between plasma and skin parameters were performed. The strongest associations were observed between changes in CRP and IL-17A and improvements in skin hydration and wrinkle reduction; however, overall correlations remained modest. Nevertheless, even subtle shifts in key regulatory mediators may meaningfully influence physiological homeostasis in healthy individuals, including target tissues such as skin and joints. These findings are consistent with evidence from psoriasis, a chronic autoimmune skin disease, where systemic elevations of IL-17A, IL-1β, TNF-α, and IL-10 are well documented [42–46].

Overall, the present data indicate that oral HA supplementation is associated with selective but biologically coherent changes in circulating immune and metabolic markers in healthy adults. Rather than inducing broad remodeling of the plasma metabolome or lipidome, HA appeared to affect specific inflammatory and immune–metabolic pathways, including IL-1β/CRP-related inflammatory status, arachidonic-acid-associated lipid signaling, and selected metabolic biomarkers such as fetuin-A and adipsin. These findings support the concept that oral HA may act as a systemic modulator of immune–metabolic balance, although the magnitude of the effect, its clinical relevance, and the underlying gut-mediated mechanisms require further validation.

Limitations and suggestions for the future

Several limitations should be considered when interpreting these findings. First, the study was conducted in a heterogeneous healthy volunteer cohort, and potential confounders such as diet, lifestyle, transient infections, physical activity, and hormonal status were not strictly controlled throughout the intervention period. This design increases real-world relevance but may also contributed to increased inter-individual variability and reduced the ability to detect subtle systemic effects. Second, xanthan gum was used as the placebo to match the sensory and physical properties of the HA-containing formulations. Although xanthan gum is a widely used food additive and the administered amount was low, it is a fermentable polysaccharide with potential effects on gut microbiota and microbial

metabolites. Therefore, the present study cannot fully distinguish HA-specific effects from differences arising from the biological activity of the xanthan gum-containing placebo. Third, biological sampling was restricted to plasma. The absence of fecal samples prevents direct assessment of microbiome-mediated mechanisms, and the lack of skin biopsies limits interpretation of how systemic biomarker changes relate to tissue-level effects. In addition, multiplex immunoassays were performed only in the placebo and SH120 groups; consequently, dose-dependent effects could not be evaluated for these protein biomarkers. Finally, the omics analyses were exploratory and require targeted validation. Future studies should combine plasma proteomics, targeted metabolite and oxylipin validation, fecal microbiome analysis, and tissue-level readouts to clarify the mechanisms by which orally administered HA influences systemic and peripheral physiology.

CONCLUSION

In conclusion, oral supplementation with HMW HA for 12 weeks was associated with selective changes in plasma inflammatory and metabolic markers in healthy adults, most notably reductions in IL-1β, CRP, arachidonic acid, fetuin-A, and adipsin. These findings suggest a modest shift toward a less inflammatory and more metabolically favorable systemic profile, consistent with a potential role of oral HA in immune–metabolic regulation. However, the effects were rather selective. Further studies using targeted mechanistic approaches and microbiome analysis are needed to confirm HA-specific effects and clarify the pathways linking oral HA intake with systemic and tissue-level outcomes.

Abbreviations

CRP – C-reactive protein, 12, 13-diHOME - Dihydroxyoctadecenoic acid, DHA - Docosahexaenoic acid, EGF – epidermal growth factor, EPA - eicosapentaenoic acid, FDR – false discovery rate, FGF2 – fibroblast growth factor 2, GLP-1 – glucagone like peptide 1, HA - hyaluronic acid, HMW – high-molecular-weight, 5-HETE - 5-hydroxyeicosatetraenoic acid, 9-HODE - 9-hydroxyoctadecadienoic acid, IFN-γ – interferon-γ, IL – interleukin, IL-1RA – interleukin-1 receptor antagonist, LC-MS - liquid chromatography–mass spectrometry, M0 – month 0, M3 – month 3, MCP-1 - Monocyte chemoattractant protein-1, PC – phosphatidylcholine, PCA - principal component analysis, PE – phosphatidylethanolamines, PGE – prostaglandin E, QC – quality control, SH60 – orally administered sodium hyaluronate at 60 mg/day, SH120 – orally administered sodium hyaluronate at 120 mg/day, sPLS - Sparse Partial Least Squares, TEWL - transepidermal water loss, TG – triglyceride, TNF-α – tumor necrosis factor α.

Declarations

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CrediT Statement

Conceptualization: Romana Šínová, Lukáš Kubala, Matěj Šimek; **Data Curation**: Romana Šínová, Vratislav Berka, Petra Ovesná; **Formal Analysis**: Lukáš Kubala, Matěj Šimek; **Investigation**: Romana Šínová, Vratislav Berka; **Methodology**: Vratislav Berka, Petra Ovesná; **Project Administration**: Lukáš Kubala, Matěj Šimek; **Supervision**: Lukáš Kubala, Matěj Šimek; **Visualization**: Romana Šínová, Vratislav Berka; **Writing - Original Draft Preparation**: Romana Šínová, Matěj Šimek; **Writing - Review and Editing**: Lukáš Kubala, Matěj Šimek

Conflicts of interest

RŠ, MŠ, and VB were employees of Contipro a.s. The company had no involvement in the study design, data collection, statistical analysis, interpretation of results, manuscript preparation, or the decision to submit the manuscript for publication. PO and LK declares no conflict of interest.

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Figures

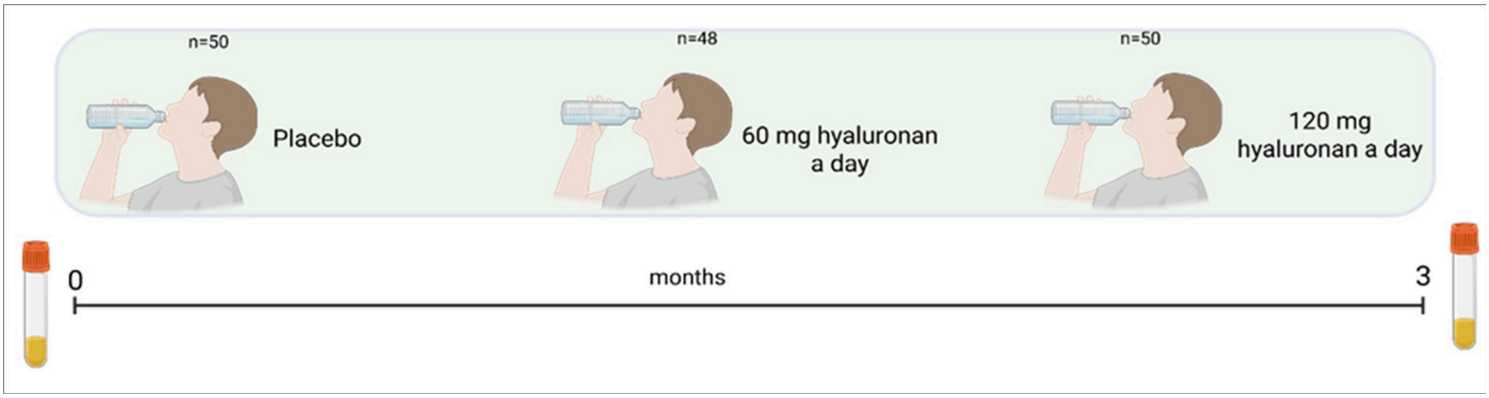


Figure 1

Study design. Plasma from healthy human donors in three experimental groups (drinking placebo, SH60 (60 mg day^{-1} HA), and SH120 (120 mg day^{-1} HA) was analysed at time 0 and after 3 months.

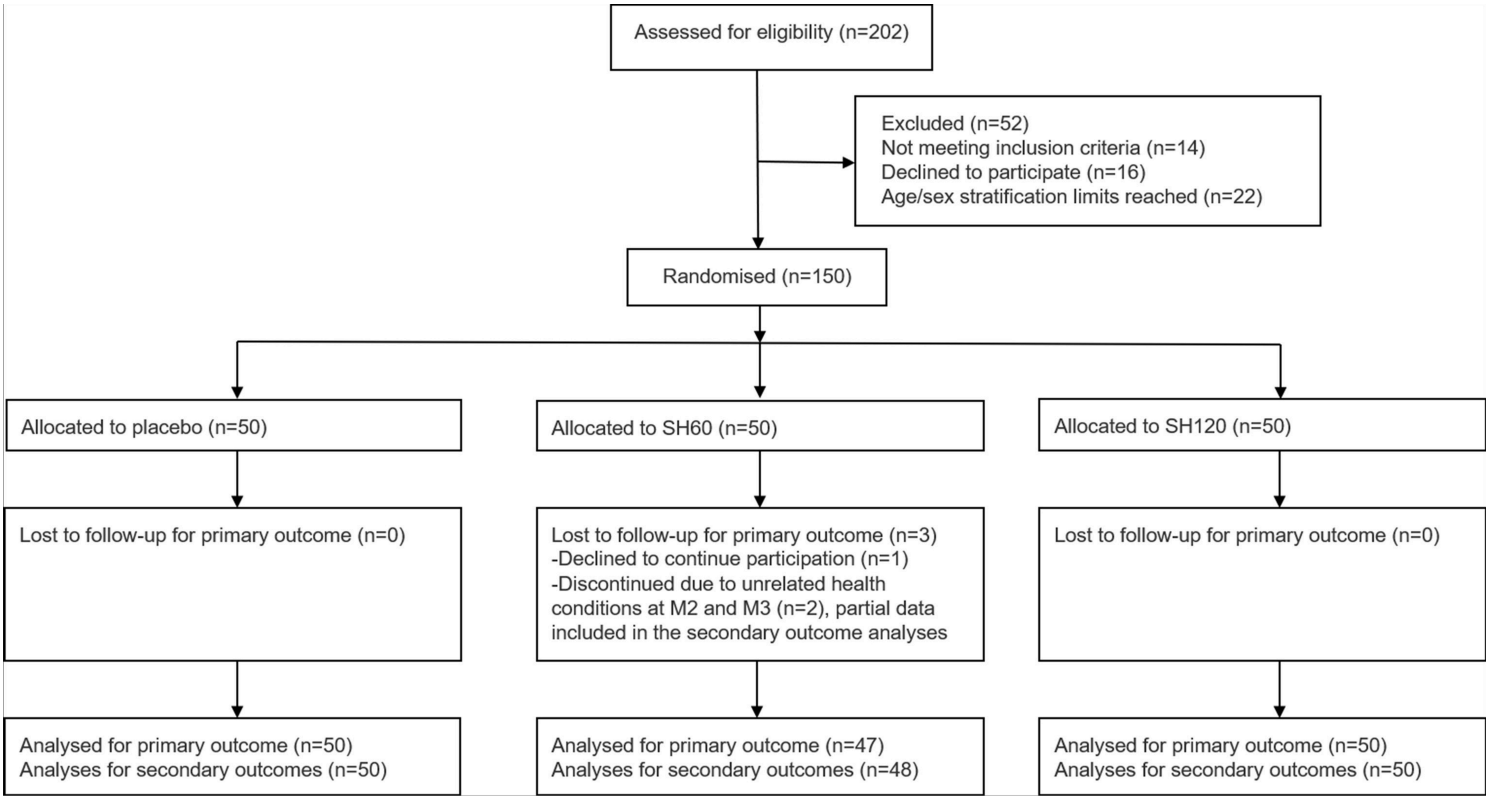


Figure 2

Participant flow diagram

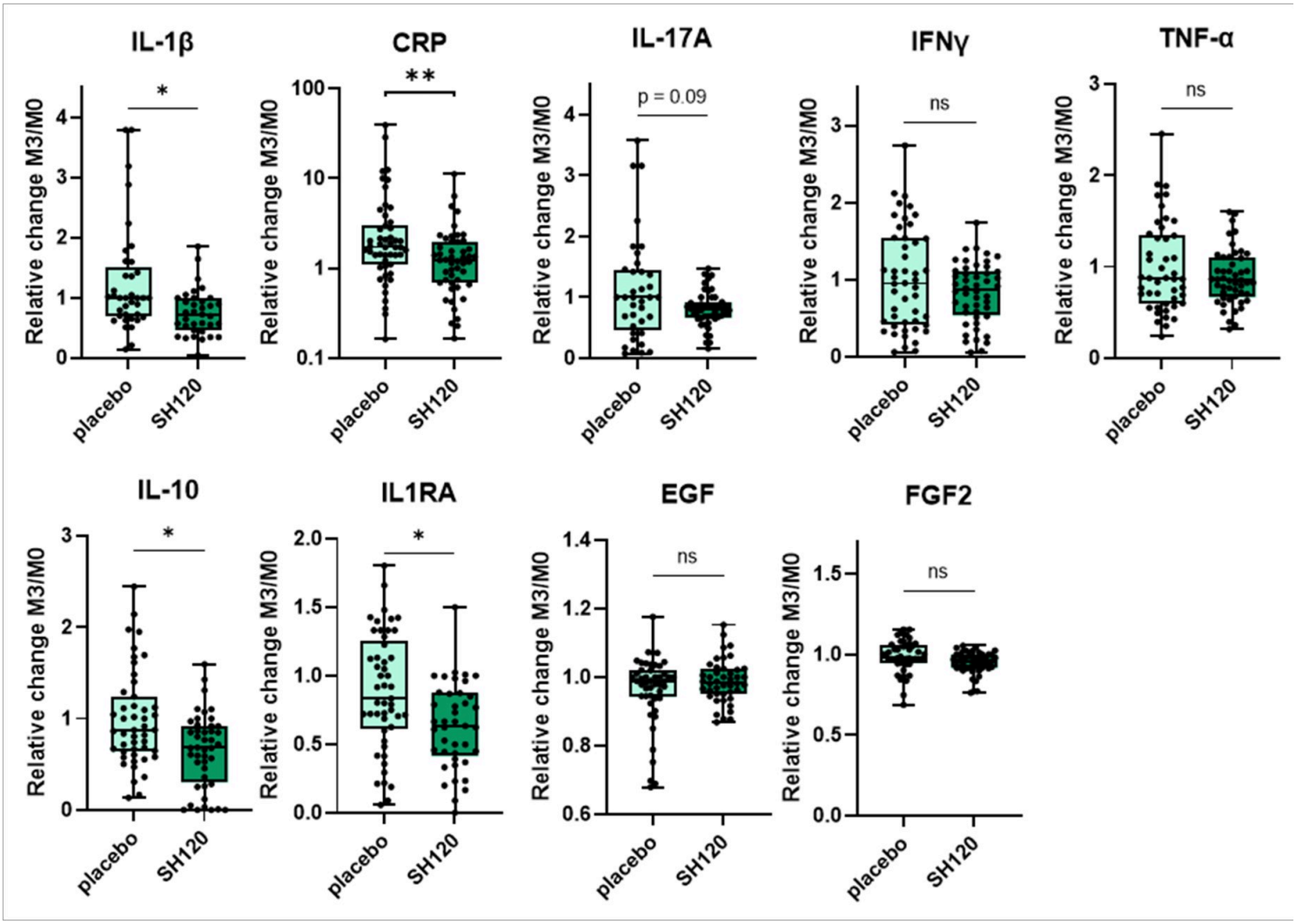


Figure 3

The levels of selected immune analytes in human plasma. Interleukine-1β (IL-1β), C-reactive protein (CRP), interleukine-17A (IL-17A), interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukine-10 (IL-10), interleukin 1 receptor antagonist (IL-1RA), epidermal growth factor (EGF), and fibroblast growth factor 2 (FGF2) were detected in placebo and SH120 groups in 0 and 3 months using multiplex assay analysis. The data are presented as the median, 25th, 75th percentile, min, max; statistical differences were tested using the Mann-Whitney test; n = 50 per group and time point, *p < 0.05, **p < 0.01 compared to the placebo group.

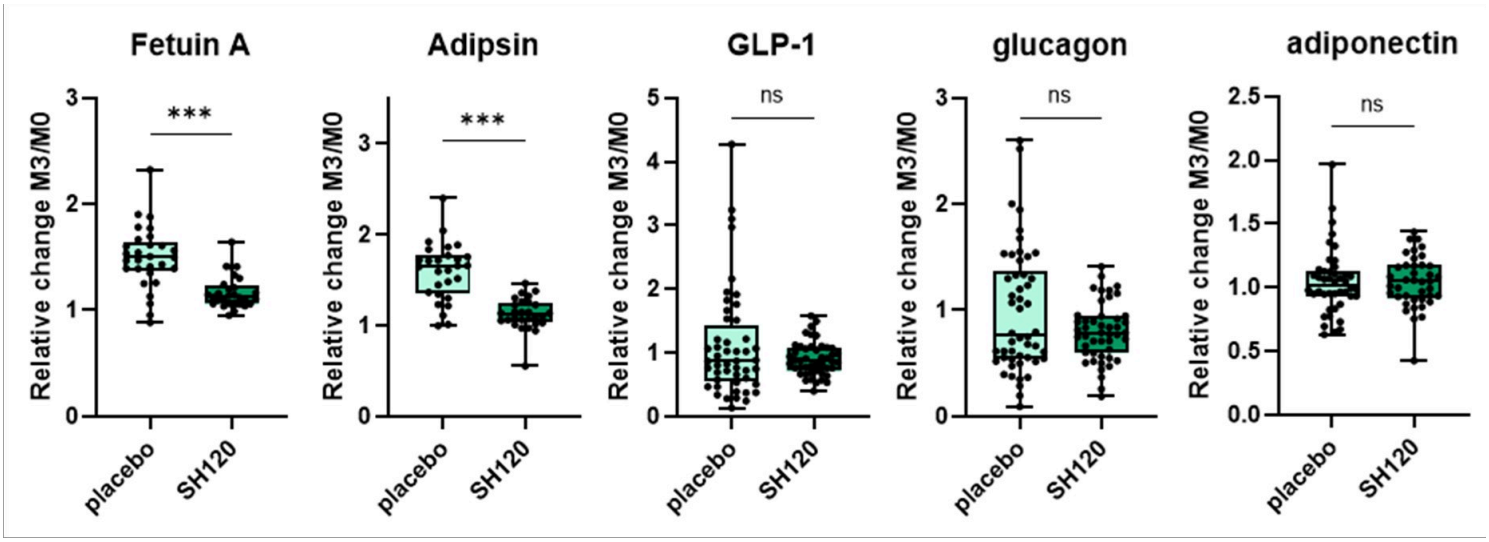


Figure 4

The levels of selected metabolic analytes in human plasma. Fetuin-A, adipsin, glucagon-like peptide-1 (GLP-1) (total), glucagon, and adiponectin were detected in placebo and SH120 groups in 0 and 3 months using multiplex assay analysis or ELISA (in case of adiponectin). The data are presented as the median, 25th, 75th percentile, min, max; statistical differences were tested using the Mann-Whitney test; n = 50 per group and time point (n = 29 for fetuin-A and adipsin), ***p < 0.001 compared to the placebo group.

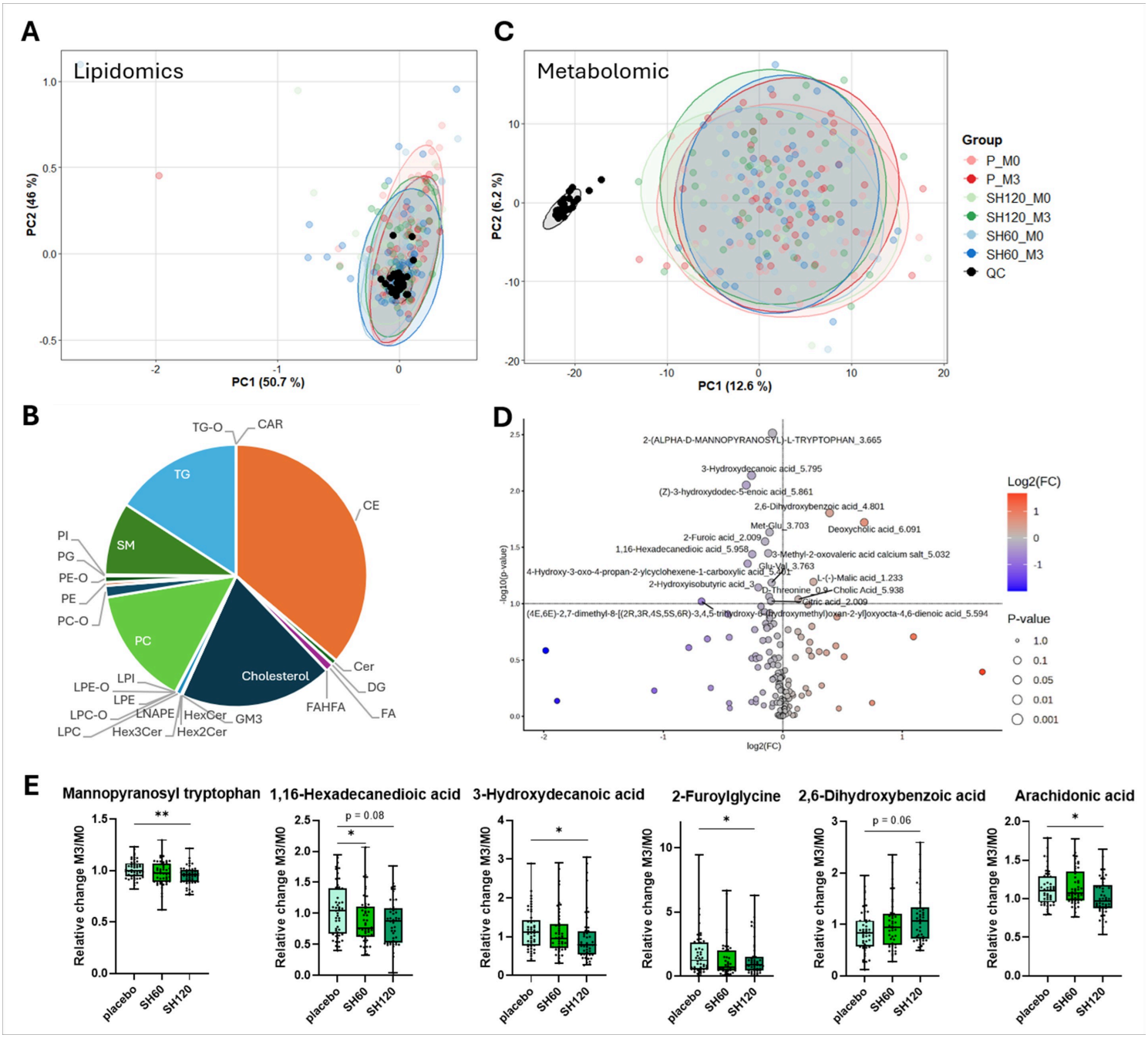
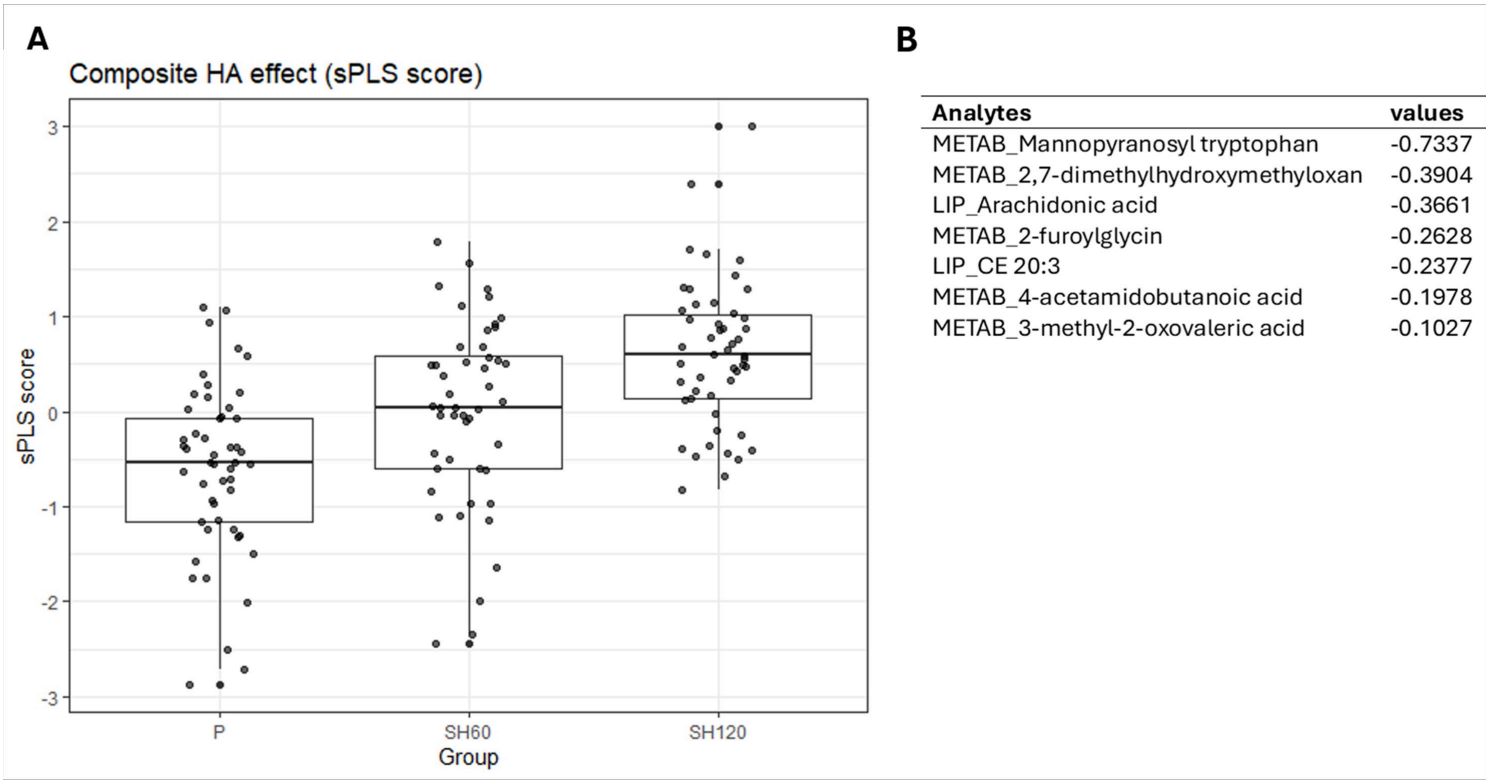


Figure 5

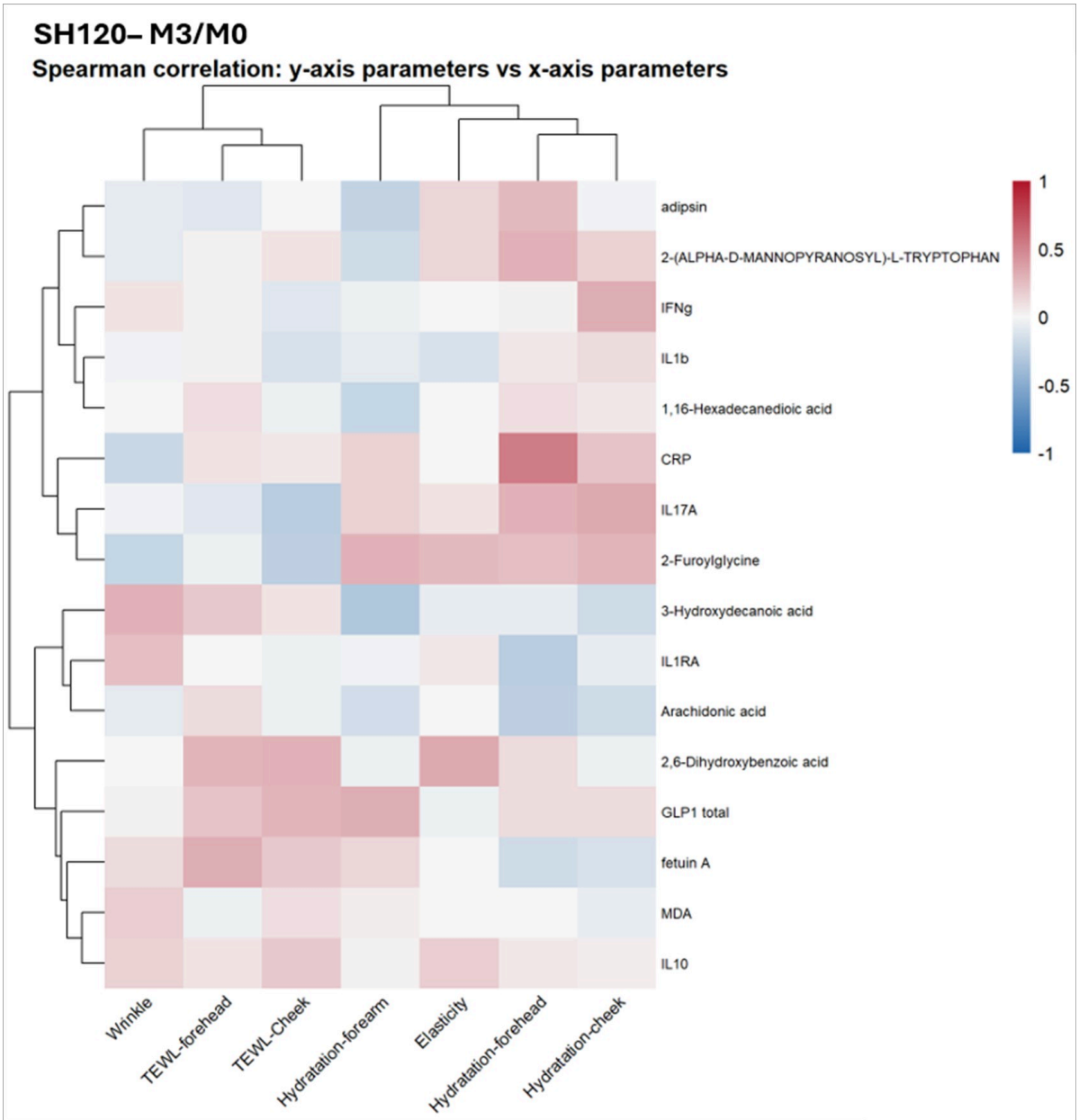
Impact of oral HA supplementation on the plasma metabolome and lipidome. A) PCA of human plasma lipidome. B) Average composition of lipid classes in the plasma. C) PCA of the plasma metabolome. D) Differences in abundances of metabolites in plasma for the SH120-placebo intergroup comparison. Statistical differences were tested using the Mann-Whitney test. E) The box plots represent M3/M0 ratios calculated from analyses performed at month 0 (M0) and at month 3 (M3) using omics methods. The data are presented as the median, 25th, 75th percentile, min, max. Statistical differences were tested using the Kruskal-Wallis test followed by Dunn's post-hoc test for pairwise comparisons; *n* = 50 per group and time point, *p < 0.05, **p < 0.01 compared to the placebo group.



Analytes	values
METAB_Mannopyranosyl tryptophan	-0.7337
METAB_2,7-dimethylhydroxymethyloxan	-0.3904
LIP_Arachidonic acid	-0.3661
METAB_2-furoylglycin	-0.2628
LIP_CE 20:3	-0.2377
METAB_4-acetamidobutanoic acid	-0.1978
METAB_3-methyl-2-oxovaleric acid	-0.1027

Figure 6

Dose-dependent effects of HA supplementation on metabolomic and lipidomic parameters A) Box plots illustrating the composite sparse Partial Least Squares (sPLS) scores across the study groups (Placebo, SH60, and SH120). The composite score exhibits a clear dose-dependent shift, transitioning from predominantly negative values in the placebo group to progressively higher scores in the HA-treated groups, with the maximum shift observed in the SH120 group. Data are presented as the median, 25th, and 75th percentiles, minimum, and maximum. B) A summary table detailing the seven selected variables (analytes) that comprise the final sPLS signature. The table lists the analytes alongside their respective directional associations and relative statistical weights (loadings), representing the variables with the strongest statistical contribution to the observed dose-response pattern.



Analytes (y parameters)	Comestics (x parameters)	S rho
MAGPIX_CRP	Hydratation (forehead)	0.5340
MAGPIX_IL-17A	Hydratation (Cheek)	0.3389
METAB_2,6-dihydroxybenzoic acid	Elasticity	0.3378
METAB_3-hydroxydecanoic acid	Hydratation (forearm)	-0.3199
MAGPIX_IL-17A	TEWL (Cheek)	-0.2859
MAGPIX_IL-1RA	Hydratation (forehead)	-0.2808

Figure 7

Associations between cosmetic parameters and plasma analytes for the SH120 group. Heatmap based on Spearman's rank correlation (details in 2.8) using hierarchical clustering for cosmetic parameters comparing selected analytes in plasma. Spearman's Rank Order Correlation was performed from M3/M0 ratios calculated from analyses at month 0 (M0) and at month 3 (M3). The strongest correlations are listed in the table below the heatmap.

Supplementary Files

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- Supplementarymaterials.docx