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Investigating the efficacy of 18-week salmon protein hydrolysate supplementation on metabolic inflammation, well-being, and cosmetic outcomes: A pilot clinical trial in healthy adults

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ABSTRACT

Background: Fatigue is a common and debilitating symptom associated with aging, which significantly reduces quality of life. Salmon protein hydrolysate (SPH) has recently been investigated for its potential benefits in improving energy levels, reducing oxidative stress, and promoting cosmetic health.

Objective: The primary objective of this proof-of-concept clinical trial was to evaluate the effects of daily low-dose SPH supplementation on energy levels, oxidative stress, inflammation, and cosmetic outcomes in healthy adults, with a focus on potential gender-specific effects.

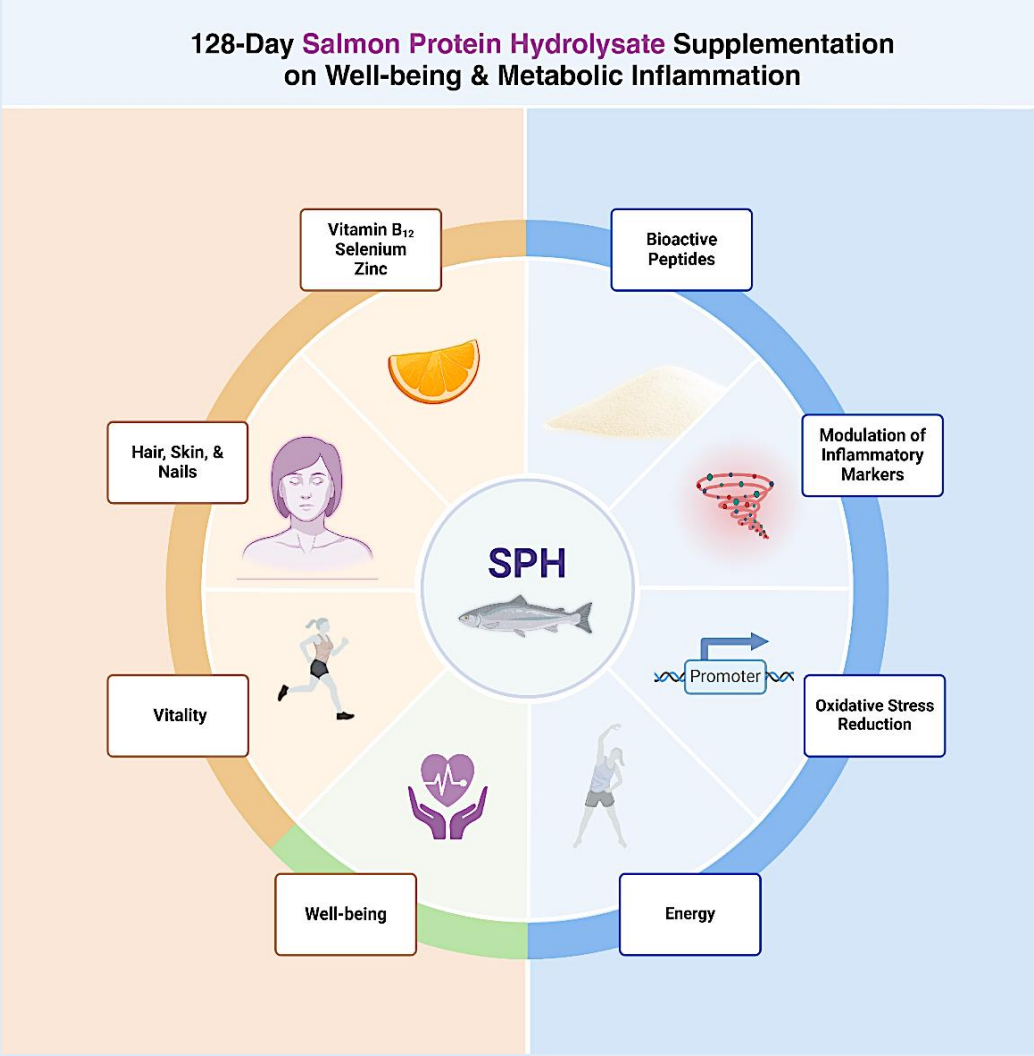
Methods: This open-label, single-arm study involved 20 participants, of which 70.6% were female. Participants consumed 4g of SPH daily for 128 days. Key outcomes measured included changes in perceived energy levels, antioxidant gene expression (HMOX1 and FTH1), free radical activity, serum cytokine levels (IL-5, IL-8, CRP, IL-2, IL-10), hematological parameters, and self-reported cosmetic outcomes. Assessments were made throughout the study to track changes over time.

Results: SPH supplementation led to a significant 10.24% improvement in perceived energy levels ($P = 0.004$), with a greater improvement observed among women (14.55%, $P = 0.008$). A 9% reduction in oxidative stress ($P < 0.001$) was accompanied by up regulation of HMOX1 and FTH1 genes by 4.09-fold and 3.77-fold, respectively. Pro-inflammatory

markers IL-5, IL-8, and CRP decreased significantly ($P < 0.001$), while anti-inflammatory markers IL-2 and IL-10 increased ($P < 0.001$). Self-reported cosmetic outcomes improved by 23.7% ($P = 0.03$), with women reporting a 34.5% enhancement in hair, skin, and nail health ($P = 0.009$).

Conclusion: Daily supplementation with low-dose SPH improved energy levels, reduced oxidative stress, and modulated inflammatory markers in healthy adults. Notably, women experienced greater improvements in both energy levels and cosmetic outcomes, suggesting SPH may be a beneficial supplement for enhancing overall well-being and cosmetic health, particularly among women.

Keywords: Salmon protein hydrolysate, oxidative stress, inflammation, fatigue, cosmetic outcomes, aging.



Graphical Abstract: Investigating the efficacy of 18-week salmon protein hydrolysate supplementation on metabolic inflammation

INTRODUCTION

Chronic diseases driven by diet and lifestyle factors are among the leading causes of death worldwide [1]. Implementing dietary and lifestyle strategies can reduce the risk of many chronic conditions [2]. Our daily habits have a cumulative impact on overall quality of life. Lifestyle habits become key modulators in preventing and treating disease, with small dietary changes having clinically significant health impacts. For instance, diets rich in healthy fats, such as those derived from certain oils and fish, are associated with reduced mortality rates from conditions like coronary heart disease [3]. Long-term, targeted dietary interventions are recognized as a means of epigenetic regulation, with significant implications for health. In the future, the relevance of dietary and lifestyle practices are particularly relevant in supporting healthy aging, a major challenge of the 21st century. With the elderly population projected to reach 33% by 2050 [4], early lifestyle interventions can slow aging and reduce disease incidence.

Fatigue is commonly associated with numerous chronic health conditions and has a significant impact on health and quality of life [5]. Additionally, fatigue is frequently reported by otherwise healthy individuals and is one of the leading concerns presented in primary care consultations [6]. Adverse dietary choices may exacerbate fatigue states, which have implications for functioning in the workplace. A prevalent nutritional deficiency is iron deficiency, which may present with fatigue and weakness without overt clinical anemia [7]. Metabolically active cells rely on sufficient iron for normal metabolic processes. As a result, iron deficiency may significantly impact muscle function, oxidative energy metabolism, the immune system, and the nervous system [8, 9]. Promoting a healthy iron metabolism is crucial for overall well-being, considering its central role in energy production.

Over the past decade, extensive research has been focused on uncovering the health benefits of fish protein hydrolysates (FPH), particularly highlighting the range of diverse bioactive peptides found within these complexes. Bioactive peptides represent a diverse group of short peptide sequences, typically composed of 2 to 20 amino acids, with the capacity to modulate various physiological processes. Recent studies have highlighted the potential of fish protein hydrolysates (FPH) in managing metabolic disorders and improving gut microbiota composition, particularly with their effects on glucose metabolism and systemic inflammation [10].

In recent years, bioactive peptides have garnered increasing attention as a promising area of research due to their therapeutic potential in disease prevention and health enhancement. FPH, a product derived from the enzymatic breakdown of fish proteins, represents a nutritionally viable dietary intervention enriched with bioactive peptides that may confer health benefits beyond basic nutritional support [11-12]. With rising consumer demand for foods offering both taste and health benefits, functional foods—products that deliver health advantages beyond basic nutrition—have become a focus of innovation [13]. Functional foods are founded on health-promoting bioactive compounds within a food matrix, delivering physiological benefits beyond basic nutrition. These foods can take many forms, from traditional foods naturally rich in bioactive ingredients to fortified products aimed at reducing disease risk in targeted populations. According to the Functional Food Center, these foods must contain bioactive compounds in safe and effective amounts that provide scientifically validated health benefits and contribute to reducing the risk of chronic diseases [14]. Atlantic salmon (*Salmo salar*) and its post-filleting raw materials contain bioactive ingredients like omega-3's EPA and DHA,

antioxidants, and bioactive peptides. Moreover, it contains high-quality protein, lipids, polysaccharides, carotenoids, vitamins, and trace minerals, including selenium [15]. Salmon contains compounds with potential cardiometabolic benefits, which may act additively or synergistically for an overall beneficial health effect [16]. Through mild enzymatic hydrolysis, raw materials left over post-filleting can be converted into value-added products suitable for human consumption while maintaining innate nutritional properties. Fish protein hydrolysates also offer significant potential for improving sustainability in aquaculture and functional food industries, owing to their bioactive and antioxidant properties [17]. Salmon's well-balanced amino acid composition provides high nutritional value, including essential amino acids often absent in plant-based proteins [18, 19]. Enzymatic hydrolysis releases peptides from their parent proteins, some of which are bioactive and capable of exerting physiological functions in vivo, depending on their primary sequence. Salmon-derived peptides consistently offer radical scavenging, antioxidant, anti-inflammatory, anti-diabetic, antihypertensive and anti-proliferative activities [18]. These bioactive peptides have the potential to improve health, mitigate disease processes, and enhance quality of life in a practical manner [12].

Previously, a 4-week fish hydrolysate supplementation was found to increase subjective sleep quality, as well as ameliorate daytime sleepiness in a double-blind, randomized controlled trial (RCT), suggesting a potential role for fish protein hydrolysates in enhancing both physical health and overall well-being [20]. Mechanistically, it may exert a role in circadian rhythm regulation [21]. Certain components of salmon hydrolysates have demonstrated anxiolytic effects, which provide preliminary support for the adjunct use of fish hydrolysates as a strategy for stress management [22]. Collagen, another abundant protein in fish-processing by-products, is not commonly incorporated into cosmetic products for its beneficial biological properties, particularly as a cosmetic anti-aging ingredient. By our mid-20's, our skin collagen content reaches its peak, thereafter, the collagen production declines by roughly 1.0%-1.5% per year, which may be accelerated by factors such as smoking and UV exposure [23-25]. Mechanistic studies have shown that fish by-product proteins can mitigate the effects of skin photoaging. Ingestion of hydrolyzed collagen can be observed in human blood as di- and tripeptides— such as Pro-Hyp, Hyp-Gly, and Gly-Pro-Hyp— which reach the dermis to modulate the resident fibroblasts [26]. Marine collagens are positioned as an optimal source of collagen for improving skin hydration, due to their high bioavailability and favorable safety profile [27, 28]. Longer-term use appears to yield more favorable effects on skin hydration and elasticity than short-term use of collagens from clinical trials.

Previously, treatment with a salmon protein hydrolysate (SPH; also known as ProGo®) demonstrated gene up-regulation for 16 human oxidative protective genes, with a concomitant down-regulation for 9 human oxidative stress genes [29]. Three genes—ferritin heavy polypeptide-1 (*FTH1*), heme oxygenase-1 (*HMOX1*), and arachidonate 12-lipoxygenase (*ALOX12*)—exhibited a notable fold-change in gene expression to a magnitude that supports enhanced health. Building on these findings, the same SPH was employed in a controlled clinical trial, where iron-deficient anemic participants received 16 grams (g) of SPH or whey protein isolate (WPI) daily for six weeks. Both SPH and WPI have a minimal iron content: less than 0.05 mg per 16.0 g serving compared to 0.3 mg per 18g serving, respectively. Participants treated with SPH displayed a statistically

significant 14% increase in hemoglobin levels ($p < 0.05$), compared to a 2% increase in the WPI group [30]. In another clinical trial, SPH at a daily intake of 16 g for 42 days, mean BMI for participants was significantly reduced by 5.9% ($p < 0.05$) compared to a WPI control [31]. Moreover, the post-supplementation results revealed significant changes in four metabolism-relevant serum biomarkers, highlighting a significant impact of the SPH on metabolic parameters. Further *in vitro* investigations have found the SPH to contain significant glucoregulatory bioactivity [32], and specific *FTH1*-modulating peptides to enhance the anti-proliferative effect of anti-androgen drugs [33-34].

The current proof-of-concept trial aimed to evaluate the efficacy of an SPH on participants vitality and quality of life with a 128-day supplementation protocol. A panel of oxidative stress-related genes and assessments of free radical activity were investigated as well as serum signatures of inflammatory cytokines and clinically relevant hematological parameters. Finally, cosmetic outcomes (subjective skin, nail and hair quality improvements) were evaluated, to determine the benefits of SPH supplementation on outward signs of aging.

MATERIALS AND METHODS

Study Design: This study was designed as a single-center, single-arm, open-label, proof-of-concept clinical trial conducted in accordance with the Declaration of Helsinki and ICH guidelines for Good Clinical Practice.

The protocol was approved by the Institutional Review Board of KGK Science Inc., London, Ontario, Canada, and all participants provided written informed consent prior to inclusion in the study. The study was conducted at KGK Science Inc., and the final follow-up data collection was completed on October 23, 2018.

Participants were enrolled based on specific inclusion and exclusion criteria. The primary objective of the study was to evaluate the change from baseline to the end of the study in self-assessed levels of vitality, as assessed by the Vitality and Quality of Life Questionnaire. Secondary objectives included changes in red blood cell count, RBC indices, hematocrit, hemoglobin levels, and other biomarkers of oxidative stress and inflammation. The intervention involved administering 4750 mg sachets of SPH dissolved in water, taken twice daily for 128 days. Compliance was monitored through regular follow-ups, and adverse events were recorded. Baseline assessments included vital signs, anthropometric measurements, and various blood tests.

Study Population: The clinical trial included 20 healthy male and female participants aged between 30 and 60 years, with a Body Mass Index (BMI) between 18.5 and 32.5 kg/m^2. Participants were required to be generally healthy, maintain their normal diet and exercise routines, and provide written informed consent prior to the study.

The study's exclusion criteria encompassed pregnancy, breastfeeding, plans to become pregnant, recent blood donation (within 30 days prior to the last study visit), known allergies to any ingredients in the intervention material, clinically significant abnormalities in physical examination at screening, recent participation in another clinical trial (within the past 30 days), cognitive impairment or inability to give informed consent, existing cardiovascular conditions or uncontrolled hypertension, type I or type II diabetes, kidney or liver disease, anemia, thyroid disorders, autoimmune conditions, immunocompromised status, and any condition that the Qualified Investigator (QI) determined could interfere with the participant's ability to complete the study or present a significant risk.

Participants were recruited from KGK Science Inc., and baseline assessments included vital signs, anthropometric measurements, and various blood tests. The study did not include a control group, and all participants received the SPH supplementation. Compliance was monitored, and adverse events were recorded throughout the study.

Nutraceutical Intervention: Participants were administered 4750 mg sachets of SPH, containing 4000 mg of collagen salmon protein hydrolysate (SPH) and 750 mg Forest Fruit Flavoring. The powder was dissolved in 100-300 mL of water and consumed within 5-10 minutes after preparation. Each 100 g of the product contained 27.9 µg of vitamin B12 and 0.77 mg of selenium, essential for overall metabolic health, as verified by Eurofins Food and Feed Testing Norway AS. Compliance was ensured by instructing participants to return all unused and open sachets to the clinic. Participants who missed a dose were directed to take it promptly after breakfast on the same day, with a strict limit of no more than two sachets within a 24-hour period.

SPH is produced through enzymatic hydrolysis of salmon by-product raw materials, sourced and manufactured by Hofseth Biocare ASA (Keiser Wilhelmsgate 24, Møre og Romsdal, 6003, Ålesund, Norway). This hydrolysate takes the form of a light-yellow powder, containing more than 95% water-soluble peptides, of which more than 25% consists of peptides derived from type I and type III marine collagen. It has a fat content of less than 0.5%, and an ash content below 2.5%. The amino acid profile includes glutamic acid (13.9 g/100 g), aspartic acid (9.4 g/100 g), glycine (14.9 g/ 100 g), proline (7.6 g/100 g), lysine (7.0 g/100 g), alanine (7.5 g/100 g), and arginine (6.9 g/100 g). The average molecular weight of the peptides, both bioactive- and non-bioactive, is approximately 3395 Dalton.

Vitality and Quality of Life Questionnaire: The Vitality and Quality of Life (VQoL) Questionnaire is a self-assessment tool designed to evaluate participants' perceived vitality and overall quality of life. It consists of 30 statements rated on a 7-point Likert scale (1 = Never to 7 = Always). Negatively worded statements are reverse scored (i.e., a decrease in score reflects an improvement, and an increase in score reflects a worsening). Participants completed the questionnaire at baseline (Day 0) and at the end of the study (Day 128), based on their experiences over a specified period. A total score was calculated for each participant by summing their responses, with higher scores reflecting a greater perceived vitality and quality of life.

Analytical Populations: Per-protocol population (PP-population): This population consists of participants who met key compliance criteria, including consuming at least 80% of the product, having no major protocol violations, and completing all required study visits. This group is used for primary efficacy analyses.

Data Analysis: Efficacy and safety analyses were primarily conducted on the PP-population, focusing on participants who adhered closely to the study protocol to evaluate the effects of salmon protein hydrolysate under optimal conditions. This approach aims to minimize variability caused by non-compliance or protocol deviations, allowing for a clearer assessment of efficacy. Secondary analyses included the Intention-to-Treat (ITT) population to provide a broader perspective on the intervention’s impact, encompassing all randomized participants and reflecting real-world scenarios.

Data normality was assessed, and non-parametric tests were applied for variables that did not meet normal distribution criteria. Missing data were handled using the last-observation-carried-forward (LOCF) approach. The significance level was set at $\alpha \leq 0.05$, with no covariate adjustments in the final statistical analysis, and no interim analyses were performed. All statistical analyses were executed using the R Statistical Software Package, Version 3.4.1 (R Core Team, 2017), on a Linux platform.

Adverse events (AEs) were summarized descriptively, with frequency tables detailing the nature, incidence, severity, and causality of AEs, categorized by body system and study group. Subgroup analyses were conducted to evaluate intervention effects based on gender and responder status, with responders defined as participants exhibiting a positive change in the primary outcome. Further analyses within the responder subgroup included secondary outcomes, such as self-assessment scores for hair, nails, and skin satisfaction. This comprehensive approach allowed for a nuanced evaluation of the intervention's efficacy and safety, balancing the insights gained under ideal conditions with broader applicability to diverse populations.

Power Analysis: The study was designed to enroll 20 participants, incorporating an estimated 20% attrition rate to account for potential dropouts. This calculation aimed to ensure that at least 16 participants would complete the study, providing an adequate sample size for preliminary analysis despite any expected loss of participants. Given the open-label design and the exploratory, proof-of-concept focus of this study, a formal sample size calculation or power analysis was not performed. This approach was intended to generate preliminary evidence on the effects of low-dose salmon protein hydrolysate without necessitating a confirmatory sample size typically required in later-phase studies. The primary outcome was assessed using a self-developed, non-validated questionnaire on perceived vitality, which aligns with the exploratory objectives of the trial. Findings from this trial are expected to inform the design of future, larger-scale trials utilizing validated instruments, rather than providing definitive conclusions. The study was single-center and conducted in Canada.

Compliance: Compliance was assessed by determining the percentage of doses taken in comparison to the expected number of doses, calculated with the following formula:

$$Product\ Compliance = \frac{number of dosages\ taken}{number of dosages\ expected} x100$$

Participants with compliance rates below 80% or above 120% received guidance to help them achieve better adherence to the study protocol.

Primary Outcome- Vitality Quality of Life (VQoL) Questionnaire: The primary outcome was assessed using the Vitality Quality of Life (VQoL) Questionnaire. Descriptive statistics, including the mean and standard deviation, were computed for total scores at baseline and the study's completion. Changes in scores were analyzed using paired Student's t-tests, with statistical significance set at $\alpha = 0.05$. The normality of the data was assessed using Q-Q plots and the Shapiro-Wilk test.

Secondary Outcome - Inflammatory Cytokine Analysis: Inflammatory cytokines, including IL-1RA, IL-4, IL-6, IL-10, IL-11, IL-13, and TGF-beta, were measured to evaluate both pro-inflammatory and anti-inflammatory immune responses. Cytokine concentrations were determined using a multi-analyte ELISArray kit (Qiagen, Toronto, ON,

Canada), which allows simultaneous analysis of multiple protein targets. Serum samples were prepared by centrifuging whole blood at 1000 g for 15 minutes within 30 minutes of collection, followed by storage at -20°C until analysis.

Secondary Outcome - Hematology: To assess the effects of the intervention, hematological parameters were analyzed, including red blood cell (RBC) count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), hematocrit, red cell distribution width (RDW), and hemoglobin levels. Changes in these parameters were compared within participants using paired Student's T-tests, with significance defined at $\alpha \leq 0.05$.

Secondary Outcome - Total ROS/RNS Free Radical Activity: Free radical activity was assessed using a ROS/RNS Assay Kit, which measures total free radicals containing oxygen or nitrogen in a sample through measurements of green fluorescence emission. The intensity of emitted fluorescence was proportional to total ROS/RNS activity, and therefore total oxidative stress burden. Serum samples from participants were utilized for this assay.

Secondary Outcome - Differential Oxidative Gene Expression: The study assessed changes in mRNA expression levels of 84 genes associated with oxidative stress, including *APOE, FTH1, GCLC, GPX1, HMOX1, NQO1,* and *SOD1*, using fold-change analysis. mRNA quantification was performed via quantitative real-time polymerase chain reaction (qRT-PCR) and normalized against the internal control gene, beta-actin (*ACTB*). Expression results were reported as relative fold-change values, calculated using the $2^{-\Delta\Delta}$Ct method, with a threshold fold-change of 2 indicating significant changes in gene expression likely to be biologically significant.

Secondary Outcome - Hair, Nails, and Skin Self-Assessment Questionnaire: The Hair, Nails, and Skin Self-Assessment Questionnaire was administered at baseline (Day 0) and again at the final study assessment (Day 128) to evaluate self-reported cosmetic outcomes. The questionnaire included seven parameters: Hair Volume, Hair Softness, Hair Shine, Hair Strength, Nail Strength, Skin Hydration, and Overall Skin Health, each rated on a 6-point Likert scale from "Greatly Satisfied" to "Greatly Dissatisfied." Lower scores indicated higher satisfaction, while higher scores denoted greater dissatisfaction. Participant responses were summed up and averaged across answered questions to generate an overall score.

RESULTS

Baseline Characteristics of Study Population: Out of 36 participants screened, 20 were enrolled in the study after providing informed consent. Sixteen participants were excluded due to not meeting eligibility criteria or withdrawing consent. Nineteen participants completed the intervention, with one participant lost to follow-up. Two participants were excluded from the per-protocol (PP) analysis: one due to stress potentially affecting the primary endpoint, and the other for non-adherence to the intervention protocol.

Compliance rates were high in the PP population, averaging 98.67% ± 4.05%. A total of 16 adverse events (AEs) were reported among 8 participants, with gastrointestinal distress, specifically constipation, being the most common, experienced by one participant. Other adverse events were varied and did not follow a consistent pattern.

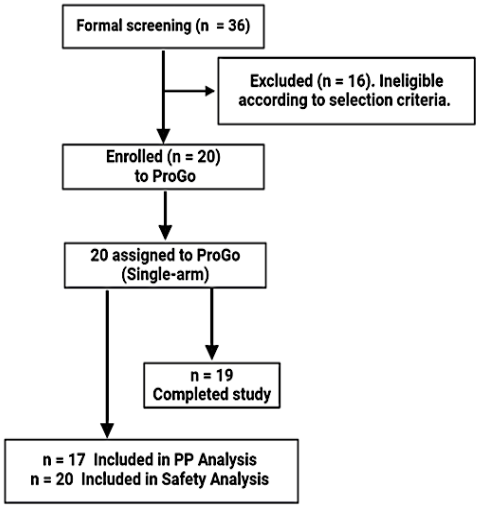


Figure 1. Participant Flow Diagram. The diagram illustrates the flow of participants through the study. A total of 36 individuals were screened, with 16 excluded due to not meeting selection criteria. Twenty participants were enrolled in the single-arm study and assigned to receive salmon protein hydrolysate. Nineteen participants completed the study, while one participant was lost to follow-up. Seventeen participants were included in the per-protocol (PP) analysis, and all 20 participants were included in the safety analysis.

The study population (see Table 1) comprised normal-weight adults with a mean age of 49.00 ± 5.88 years and a mean BMI of 25.94 ± 3.84 kg/m². The cohort was predominantly female (70.6%) with males representing 29.4% and included participants from diverse ethnic backgrounds: 52.9% Western/Eastern European White, 17.7% East/Southeast Asian, 17.7% South American, 5.9% African or African American, and 5.9% Native American. Key baseline vital signs showed a mean systolic/diastolic blood pressure of 117.73 ± 9.98 / 74.82 ± 7.92 mmHg, a mean heart rate of 65.39 ± 5.33 bpm, and average waist and hip circumferences of 87.24 ± 9.74 cm and 100.87 ± 8.38 cm, respectively.

Table 1. Baseline Demographics, Anthropometrics, and Vital Signs of the Study Population (n = 17).

Characteristic	**Mean ± SD / n (%)**
Age (years)	49.00 ± 5.88
Gender [n (%)]	
- Female	12 (70.6%)
- Male	6 (29.4%)
Ethnicity [n (%)]	
- African or African American	1 (5.9%)
- East Asian or South-east Asian	3 (17.7%)
- Hispanic or Latino	0 (0%)
- South American	3 (17.7%)
- Western or Eastern European White	9 (52.9%)
- Native American	1 (5.9%)
Systolic-/Diastolic Blood Pressure	117.73 (± 9.98)/ 74.82 (± 7.92) mmHg
Heart Rate	65.39 ± 5.33 bpm
Weight (kg)	71.25 ± 14.93
Height (cm)	165.31 ± 10.89
BMI (kg/m²)	25.94 ± 3.84
Waist Circumference (cm)	87.24 ± 9.74
Hip Circumference (cm)	100.87 ± 8.38

Table 2 provides an overview of the baseline hematological and clinical chemistry profiles of the study participants. Key parameters include Hemoglobin Concentration (g/L), Red Cell Distribution Width (RDW, %), Mean Corpuscular Volume (MCV, fL), Glycated Hemoglobin (HbA1c, %), and Fasting Plasma Glucose (FPG, mmol/L). The values, presented as mean ± standard deviation (SD), represent the participants' initial hematological status and glycemic control prior to the intervention.

Table 2. Baseline Hematological and Clinical Chemistry Parameters of the Study Population**.**

Parameter	Mean ± SD
Hemoglobin Concentration (g/L)	133.94 ± 8.48
Red Cell Distribution Width (%)	13.33 ± 0.49
Mean Corpuscular Volume (fL)	89.47 ± 3.76
Glycated Hemoglobin (HbA1c) (%)	5.36 ± 0.26
Fasting Plasma Glucose (mmol/L)	5.15 ± 0.4

Primary Outcome– Vitality and Quality of Life (VQoL): Study participants that consumed SPH daily for 128 days exhibited a statistically significant increase in self-assessed vitality (Table 3; p = 0.004). This change corresponds to a mean relative percentage improvement of approximately 10.24% from baseline to the study's conclusion.

Table 3. Self-Assessed Vitality in the Per-Protocol Population (n = 17) as measured by the VQoL Questionnaire Before and After 128 Days of SPH Supplementation.

Study day	Mean ± SD (n)	P-value
Baseline (Day 0)	144.75 ± 23.33 (20)	
End of Study (Day 128)	159.58 ± 18.88 (19)	
Change	13.89 ± 20.49 (19)	0.004

[1] n = number; SD = standard deviation; Min = minimum; Max = maximum; Paired Student's T-test was carried out to calculate the p-value.

Figure 2 illustrates the changes in VQoL scores, highlighting a statistically significant increase in self-assessed vitality from baseline to the end of the study. Subgroup analysis by gender revealed a non-significant mean score increase of 2.0 ± 4.3 (p = 0.36) in males. In contrast, females experienced a significant mean increase of 20.42 ± 22.03 points (p = 0.008), corresponding to an approximate 14.55% relative increase in total perceived energy levels from baseline to the study's conclusion.

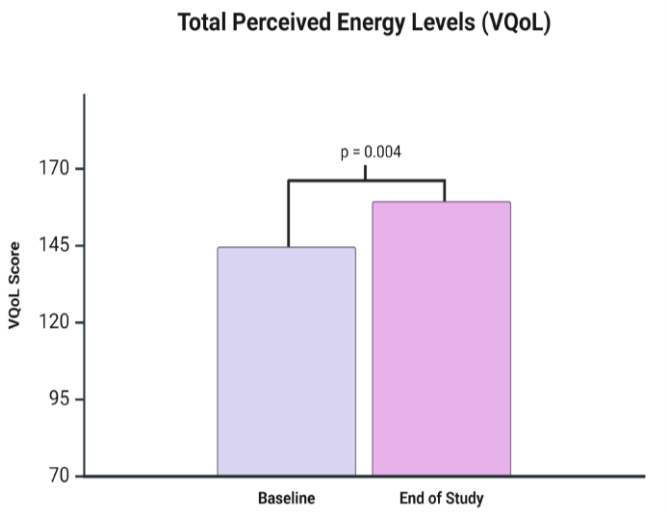


Figure 2. Vitality and Quality of Life Scores in Per-Protocol Population Over 128 Days with SPH. The bar graph illustrates a significant rise in VQoL scores from baseline to the study's conclusion, highlighting the positive effect of SPH supplementation on perceived energy levels.

VQoL Assessment – Individual Item Analysis: After 128 days of SPH supplementation, participants in the PP-population reported significant improvements in select questions from the Vitality Quality of Life (VQoL) questionnaire. As shown in Figure 3, these are the items with the greatest mean score improvements, highlighting enhancements in energy to accomplish tasks, concentration, and overall enthusiasm, underscoring the intervention's positive impact on daily life and well-being. Figure 4 highlights reductions in negatively impacting quality of life indicators, where reductions reflect decreases in symptoms such as fatigue, irritability, lack of motivation, and sluggishness.

Significant improvements were observed in energy and vitality ($p = 0.02$), and alertness ($p = 0.03$), as well as a reduction in the midday slump ($p = 0.01$) and better sustained energy levels throughout the day ($p = 0.006$) There was also a decrease in difficulty engaging in vigorous activities ($p = 0.03$), a reduction in inactivity during the day ($p = 0.05$), less listlessness ($p = 0.007$), and more stable and predictable energy levels ($p = 0.02$).

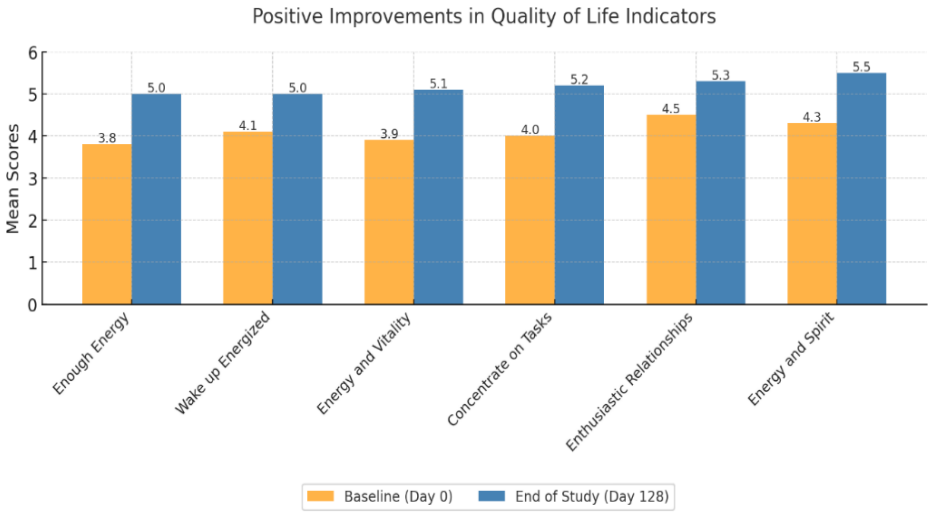


Figure 3. Improvements in Positive Quality of Life Indicators This figure shows the statistically significant improvements from baseline to the end of study in positively phrased questions from the Vitality and Quality of Life (VQoL) Questionnaire, where higher scores indicate better outcomes. The data highlights increase in energy, enthusiasm in relationships, concentration, and overall vitality.

Additional significant improvements were seen for enthusiasm and sense of engagement in relationships ($p = 0.002$), along with a reduction in tendency towards irritability ($p < 0.001$). Further enhancements included improved task initiation ($p < 0.001$), reduced sluggishness in thought processes ($p = 0.002$), and improved concentration on tasks ($p = 0.016$).

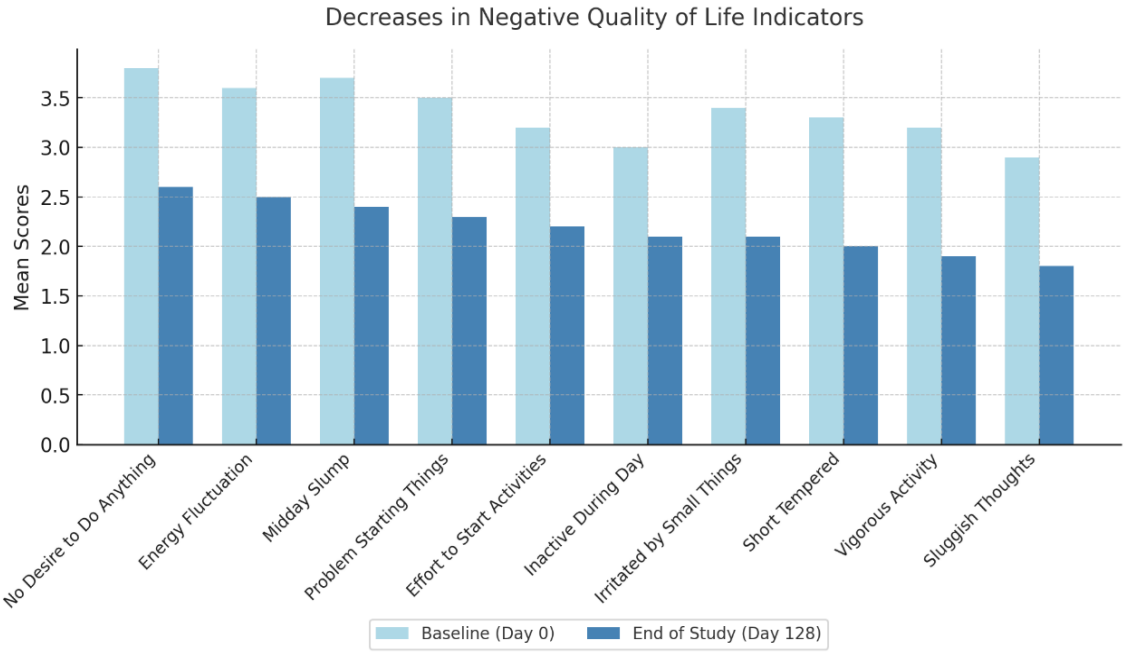


Figure 4. Decreases in Negative Quality of Life Indicators. This figure displays the mean scores for negatively phrased quality of life indicators at baseline (Day 0) and after 128 days of supplementation. Lower scores at Day 128 reflect reductions in negative symptoms such as lack of desire, energy fluctuation, irritability, and midday slump, indicating improved well-being over the course of the study.

Finally, participants experienced reductions in mild insomnia symptoms ($p = 0.04$), a decrease in waking up tired ($p = 0.02$), and an increase in waking up feeling energized ($p = 0.006$).

Secondary Endpoint – Red Blood Cell Parameters: The RBC parameters were evaluated at baseline and following 128 days of supplementation with SPH. As detailed in Table 4, the analysis revealed no statistically significant changes in mean RBC counts, MCV, MCH, MCHC, or hematocrit. However, a significant decrease in mean RDW ($p < 0.001$) was observed from baseline to Day 128. This reduction in RDW is particularly noteworthy, as elevated RDW is an early marker of iron-deficiency anemia [31]. The observed decrease suggests an improvement in RBC size homogeneity, indicating a potential normalization of red blood cell size distribution. The hemoglobin levels were assessed at baseline and at the end of the study (Day 128) as shown in Table 4. The analysis revealed a modest increase in mean hemoglobin levels from 133.94 ± 8.48 to 136.35 ± 12.11, with a mean change of 2.41 ± 6.54. However, this change was not statistically significant ($p = 0.15$).

Table 4. Red Blood Cell Parameters at Baseline and End of Study.

Parameter	Baseline (Mean ± SD)	End of Study (Mean ± SD)	Mean Change (Mean ± SD)	p-value
RBC	4.52 ± 0.33	4.58 ± 0.21	0.08 ± 0.23	0.11
MCV	89.47 ± 3.76	88.9 ± 3.34	-1.1 ± 2.36	0.49
MCH	29.66 ± 1.17	29.93 ± 1.25	0.09 ± 0.77	0.61
MCHC	332.18 ± 9.19	336.8 ± 7.4	4.65 ± 12.9	0.20
Hematocrit	0.4 ± 0.03	0.41 ± 0.03	0 ± 0.02	0.49
RDW	13.36 ± 0.46	12.56 ± 0.7	-0.79 ± 0.51	< 0.001
Hemoglobin	133.94 ± 8.48 (17)	136.35 ± 12.11 (17)	2.41 ± 6.54 (17)	0.15

RBC: Red Blood Cell Count (x10^{12}/L); MCV: Mean Corpuscular Volume (fL); MCH: Mean Corpuscular Hemoglobin (pg); MCHC: Mean Corpuscular Hemoglobin Concentration (g/L); Hematocrit (L/L); RDW: Red Cell Distribution Width (%).

Red Blood Cell Parameter Subgroup Analysis – Gender-Based Stratification: In males, hemoglobin levels increased significantly (p = 0.05), while RDW decreased significantly in both males and females (p < 0.001 for females) (Figure 5).

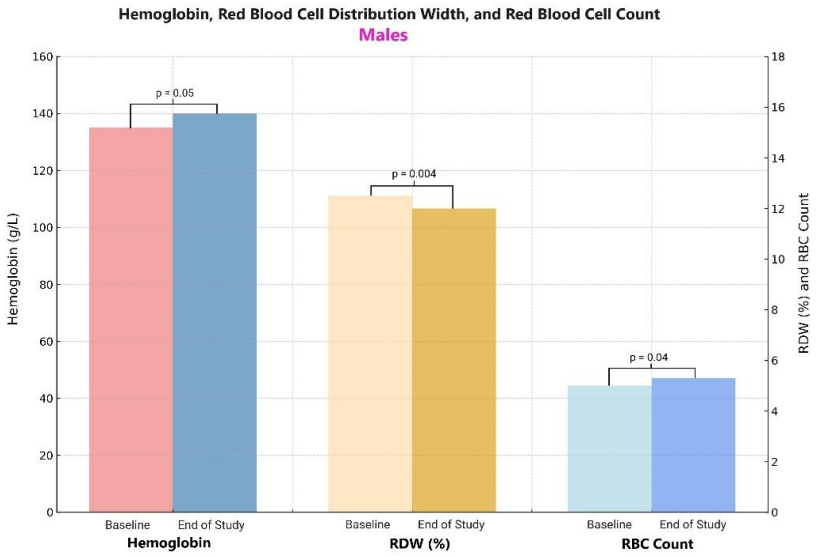


Figure 5. Hemoglobin, RDW (%), and RBC Count in Male Per-Protocol Population (n = 5). After 128 days of SPH supplementation, hemoglobin levels increased by 7.0 g/L (p = 0.05), RDW percentage decreased by 0.8% (p = 0.004), and RBC count increased by 0.21 × 10^{12}/L (p = 0.04), suggesting improved hemoglobin concentration, red cell uniformity, and erythropoiesis in the male subgroup.

Furthermore, the RBC count in males showed a statistically significant increase of 0.21 × 10^{12}/L ± 0.16 (p = 0.04), indicating that the intervention had a statistically significant positive effect on erythropoiesis in the male subgroup (Figure 5). In females (PP-population; n = 12), only the RDW showed a significant decrease of -0.91 % (± 0.25; p < 0.001).

Secondary Endpoint – Antioxidant Gene Systems: By Day 128 of SPH supplementation, the expression levels of antioxidant-related genes were analyzed, revealing significant changes in the expression of seven genes (detailed in Table 5).

Table 5. Fold-Change in Antioxidant Gene Expression with Significant Changes on Day 128 (n = 17).

Gene	Function	Fold-change Day 128 (Mean ± SD)	Median (Min - Max)
APOE	Oxidative Stress modulation	2.61 ± 0.22	2.61 (2.22-2.93)
FTH1	Antioxidant defense	3.77 ± 0.39	3.83 (2.77-4.38)
GCLC	Glutathione Synthesis	2.68 ± 0.34	2.58 (2.08-3.17)
GPX1	Antioxidant defense	2.95 ± 0.29	2.96 (2.45-3.35)
HMOX1	Antioxidant defense	4.09 ± 0.22	4.15 (3.72-4.48)
NQO1	Detoxification	3.11 ± 0.18	3.14 (2.81-3.39)
SOD1	Radical detoxification	3.06 ± 0.19	3.07 (2.78-3.49)

n = number; SD = standard deviation; Min = minimum; Max = maximum.

Notably, genes *HMOX1* and *FTH1* (see Figure 6*)*, exhibited the largest shift in mRNA expression, almost reaching a four-fold change increase, with remaining genes showing a fold increase greater than 2.5. This up regulation suggests a potential reduction in oxidative stress, supported by a significant 9% decrease in ROS/RNS free radical activity ($p \leq 0.001$).

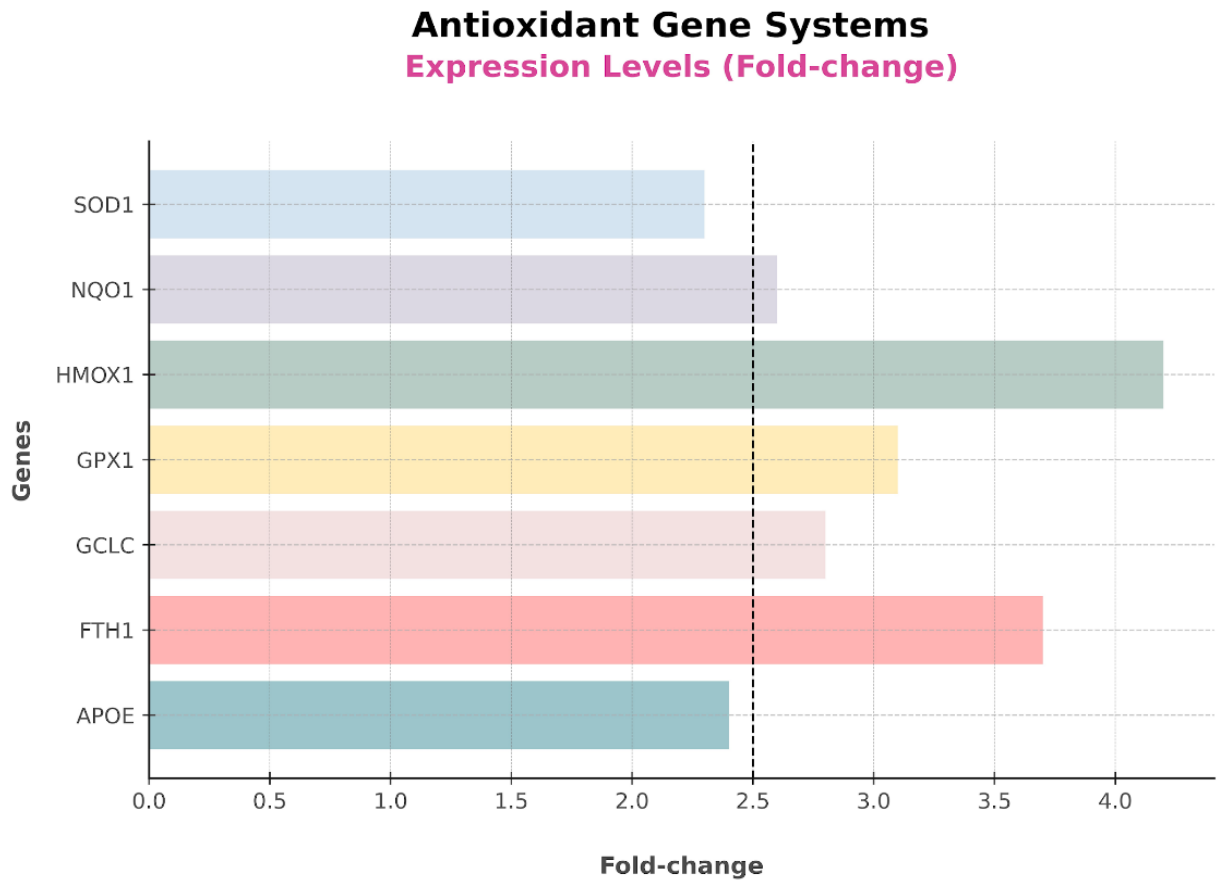


Figure 6. Differential Expression of Specific Antioxidant Genes. This figure depicts the differential expression of antioxidant genes, with fold changes above 2.5 indicating a significant up regulation in response to SPH supplementation.

Secondary Endpoint – Total Reactive Oxygen and Nitrogen Species Activity: SPH supplementation significantly reduced total ROS/RNS free radical activity, indicating a decrease in systemic oxidative stress (Table 6).

Table 6. Total ROS/RNS Activity at Baseline and Day 128 in Per-Protocol Population (n = 17).

Study Day	Mean ± SD (n)	P-value
Baseline (Day 0)	1.46 ± 0.13 (17)	
End of Study (Day 128)	1.33 ± 0.15 (17)	
Change	-0.13 ± 0.11 (17)	< 0.001

n = number; SD = standard deviation; Min = minimum; Max = maximum; Paired Student's T-test was carried out to calculate the p-value.

Figure 7 shows a significant reduction in ROS/RNS activity, with mean levels decreasing significantly from baseline to final study assessment ($p < 0.001$), reflecting a notable decrease in oxidative stress.

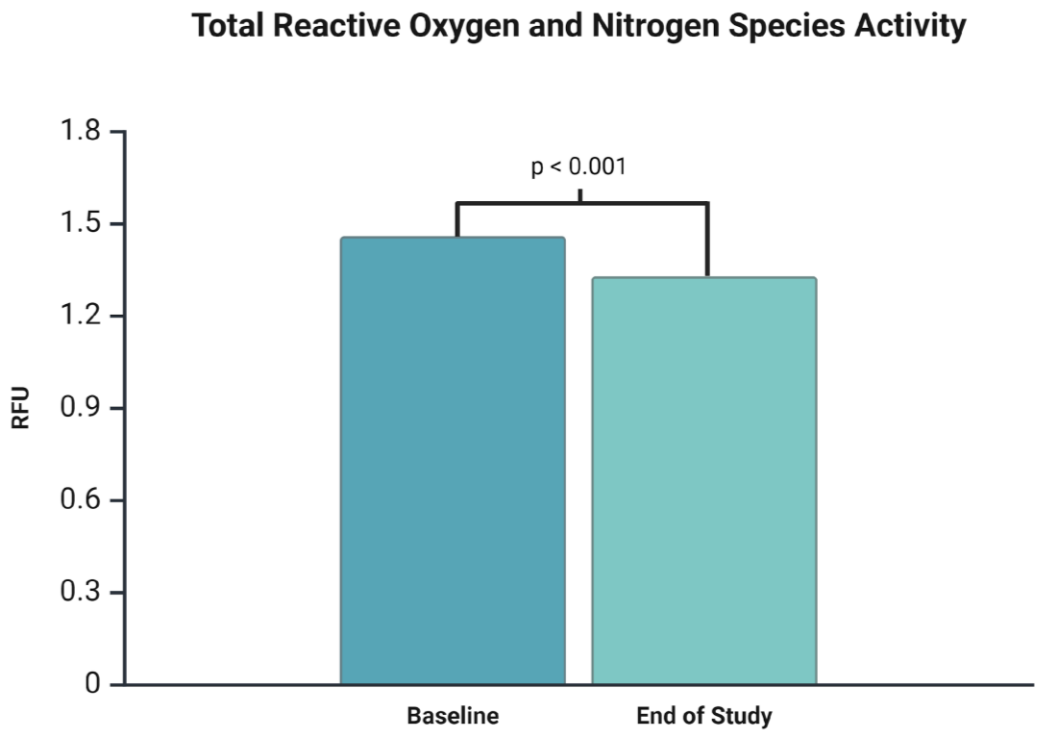


Figure 7. Average Total Free Radical Activity in the Per-Protocol Group (n = 17). This figure illustrates the mean total activity of reactive oxygen species (ROS) and reactive nitrogen species (RNS), expressed in relative fluorescence units (RFUs), at baseline and on Day 128 of the study.

Secondary Endpoint – Inflammatory Biomarkers: The study examined the changes in a panel of cytokines and C-reactive protein (CRP) from baseline to Day 128, as detailed in Table 7. Significant alterations were observed in inflammatory biomarkers, displaying an overall shift towards an anti-inflammatory phenotype.

Table 7. Changes in Inflammatory Biomarkers Following 128 Days of SPH Supplementation (n = 17). The table shows mean concentrations of key inflammatory biomarkers at baseline (Day 0) and at the end of the study (Day 128) in the per-protocol population.

Cytokine	Function	Study Day	Mean ± SD (n)	p-value
IL-1 α (pg/mL)	Inflammatory response	Baseline (Day 0)	0.47 ± 0.19 (17)	
		End of Study	0.48 ± 0.19 (17)	
		Change (Δ)	0.01 ± 0.06 (17)	0.45
IL-2 (pg/mL)	T-cell activation	Baseline (Day 0)	11.28 ± 1.45 (17)	
		End of Study	12.4 ± 1.61 (17)	
		Change (Δ)	1.12 ± 0.92 (17)	<0.001
IL-4 (pg/mL)	Immune regulation	Baseline (Day 0)	0.31 ± 0.1 (17)	
		End of Study	0.3 ± 0.1 (17)	
		Change (Δ)	0 ± 0.03 (17)	0.77
IL-5 (pg/mL)	Eosinophil activation	Baseline (Day 0)	0.52 ± 0.16 (17)	
		End of Study	0.3 ± 0.1 (17)	
		Change (Δ)	-0.2 ± 0.11 (17)	<0.001
IL-6 (pg/mL)	Inflammatory response	Baseline (Day 0)	10 ± 1.92 (17)	
		End of Study	9.93 ± 1.9 (17)	
		Change (Δ)	-0.06 ± 0.3 (17)	0.39
IL-8 (pg/mL)	Neutrophil recruitment	Baseline (Day 0)	8.84 ± 1.99 (17)	
		End of Study	7.59 ± 1.89 (17)	
		Change (Δ)	-1.25 ± 0.96 (17)	<0.001
IL-10 (pg/mL)	Anti-inflammatory response	Baseline (Day 0)	2.36 ± 0.88 (17)	
		End of Study	3.68 ± 1.13 (17)	
		Change (Δ)	1.31 ± 0.98 (17)	<0.001
IL-12 (pg/mL)	T-/NK-cell activation	Baseline (Day 0)	33.53 ± 7.07 (17)	
		End of Study	33.39 ± 7.24 (17)	
		Change (Δ)	-0.14 ± 0.93 (17)	0.54
IL-13 (pg/mL)	Allergic response	Baseline (Day 0)	52.29 ± 9.99 (17)	
		End of Study	52.26 ± 9.77 (17)	
		Change (Δ)	-0.03 ± 1.37 (17)	0.93
CRP (mg/L)	Inflammatory response	Baseline (Day 0)	1.98 ± 0.7 (17)	
		End of Study	1.72 ± 0.69 (17)	
		Change (Δ)	-0.27 ± 0.18 (17)	<0.001

Legend: n = number of participants; SD = standard deviation; Δ (Change) represents the difference between baseline (Day 0) and end of study (Day 128) values; p-values were calculated using a Paired Student's T-test. P-values < 0.05 were considered statistically significant.

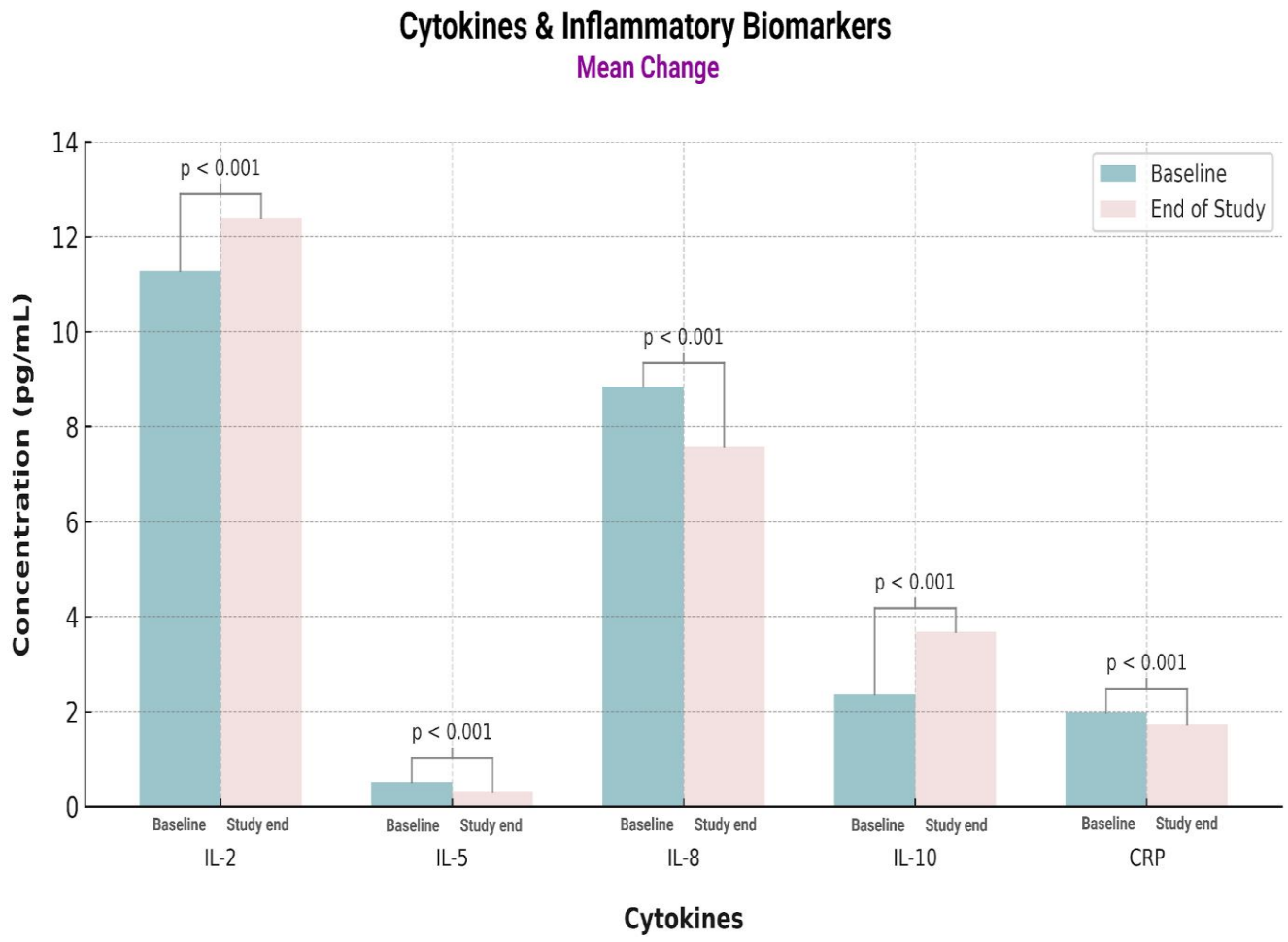


Figure 8. Average Changes in Cytokine and Biomarker Levels. This figure illustrates the mean concentrations of cytokines and biomarkers measured at baseline (Day 0) and at the conclusion of the study (Day 128) for the per-protocol population (n = 17). Statistically significant changes observed over the 128-day period are emphasized, providing insights into the dynamic shifts in these immune and biological markers during the study.

IL-2 and IL-10 levels increased by 1.12 ± 0.92 pg/mL and 1.31 ± 0.98 pg/mL (both $p < 0.001$; Figure 8), indicating heightened immune activation. In contrast, IL-5, IL-8, and CRP decreased by -0.2 ± 0.11 pg/mL, -1.25 ± 0.96 pg/mL, and -0.27 ± 0.18 mg/L (all $p < 0.001$), suggesting reduced inflammation. Other cytokines, such as IL-1 α, IL-4, IL-6, IL-12, and IL-13, showed no significant changes ($p > 0.05$).

Secondary Endpoint – Glycemic Control*:* The analysis of HbA1c levels in the per-protocol population (n = 17) revealed a statistically significant increase from baseline, with a mean change of 0.09% (SD 0.13%; $p = 0.01$). In contrast, fasting plasma glucose (FPG) levels showed a decrease from baseline (see Figure 9), with a mean change of -0.19 mmol/L (SD 0.39 mmol/L; $p = 0.06$), indicating a trend towards lower glucose levels, though not reaching conventional thresholds for significance. Overall, these results demonstrate a statistically significant increase in HbA1c levels and a nearly significant reduction in FPG levels over the study period.

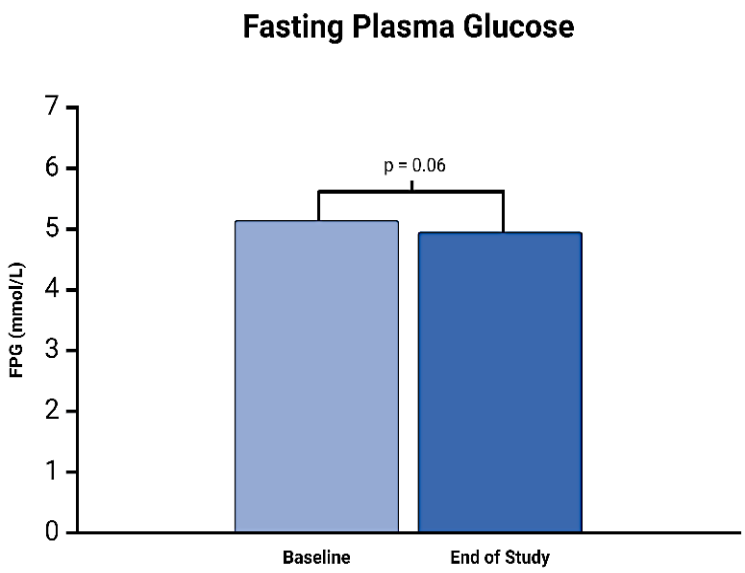


Figure 9. Reduction in Fasting Plasma Glucose (FPG) Levels on Day 128. The figure shows the decrease in FPG levels from baseline (Day 0) to Day 128 in the per-protocol population (n = 17). The average reduction was -0.19 mmol/L (SD 0.39 mmol/L; p = 0.06), indicating a trend towards lower fasting glucose levels over the study period.

Glucose Marker Subgroup Analysis – Gender-Based Stratification*:* The mean change in HbA1c for males (PP population; n = 5) over the study period was an increase of 0.2% (± 0.12; p = 0.02). A modest non-significant numerical FPG mean reduction of 0.1 mmol/L (± 0.48) was seen in the same male subgroup.

In the female subgroup (n = 12), FPG decreased by 0.23 mmol/L (±0.36), with a p-value of 0.05 indicating statistical significance. Mean HbA1c for this subgroup was non-significant, with a change of 0.04 (± 0.11; p = 0.21).

Secondary Endpoint – Self-Assessment of Hair, Nails, and Skin Quality*:* After 128 days of SPH supplementation, participants in the per-protocol population (n = 17) demonstrated a significant improvement in hair, nails, and skin self-assessment scores (Table 8). The questionnaire uses a scoring scale from 1 to 6, with lower scores signifying higher satisfaction and a more favorable perception of hair, nails, and skin condition, whereas higher scores denote increased dissatisfaction.

Table 8. Hair, nails, and skin self-assessment questionnaire scores after 128 days of supplementation with SPH.

Study Day	Mean ± SD (n)	P-value
Baseline (Day 0)	3.17 ± 1.03 (17)	
End of Study (Day 128)	2.42 ± 0.89 (17)	
Change	-0.75 ± 1.27 (17)	0.03

The mean questionnaire score decreased from 3.17 ± 1.03 to 2.42 ± 0.89, a 23.7% relative improvement in perceived hair, nails, and skin condition (p = 0.03; Figure 10), indicating an overall perceived improvement among participants.

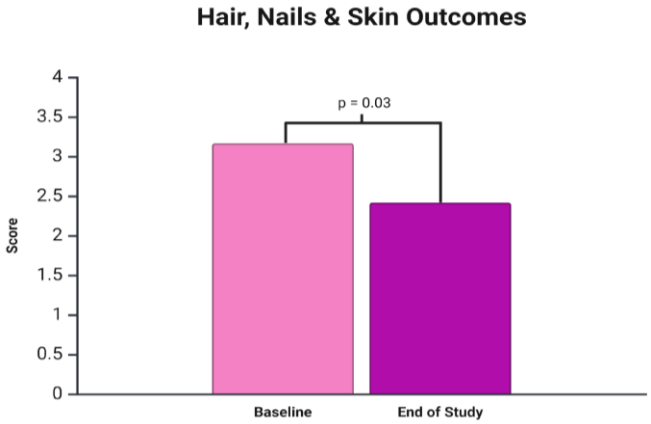


Figure 10. Changes in Hair, Nails, and Skin Satisfaction Scores in Per-Protocol Population (n = 17). This figure displays the self-assessed satisfaction scores for hair, nails, and skin quality from baseline to the study's conclusion. Lower scores indicate an improvement in perceived hair, nails, and skin quality.The gender-based subgroup analysis revealed differing responses to SPH supplementation. In males, the mean score slightly increased from 2.57 ± 0.8 at baseline to 2.83 ± 0.66 at the end of the study, showing a mean change of 0.26 ± 0.43, which was not statistically significant (p = 0.25; Table 9). Conversely, females exhibited a significant improvement, with mean scores decreasing from 3.42 ± 1.05 at baseline to 2.25 ± 0.95 at the end of the study.

Table 9. Gender-Based Subgroup Analysis of Hair, Nails, and Skin Scores After 128 Days.

Study Day	Group	Mean ± SD (n)	P-value
Baseline	Male (n = 5)	2.57 ± 0.8 (5)	
End of Study	Male (n = 5)	2.83 ± 0.66 (5)	
Change	Male (n = 5)	0.26 ± 0.43 (5)	0.25
Baseline	Female (n = 12)	3.42 ± 1.05 (12)	
End of Study	Female (n = 12)	2.25 ± 0.95 (12)	
Change	Female (n = 12)	-1.17 ± 1.28 (12)	0.009

The mean change of -1.17 ± 1.28 in females was statistically significant (p = 0.009), indicating a gender difference in response to the supplementation, reflecting a 34.5% mean relative improvement (Figure 11). The self-reported improvements in cosmetic outcomes were predominantly driven by the female participants in the study population.

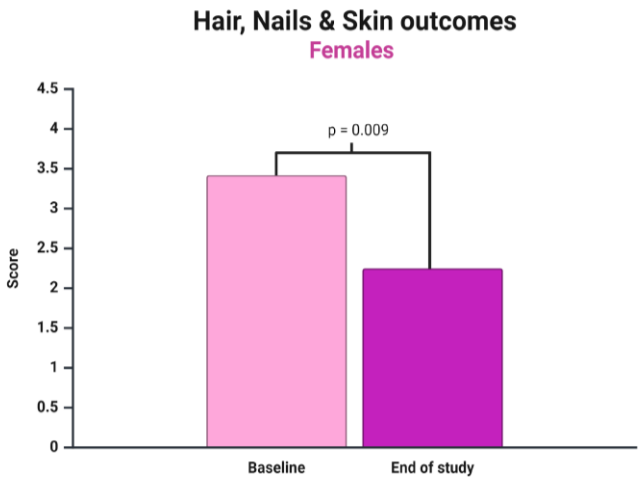


Figure 11. Hair, Nails, and Skin Satisfaction Scores in Female Population (n = 12). This figure depicts changes in self-assessed satisfaction scores for hair, nails, and skin from baseline to the study's conclusion in the female per-protocol population.

Vital Signs and Clinical Chemistry: Vital sign measurements and clinical chemistry parameters remained within normal laboratory ranges for the duration of the trial.

Adverse Events: A total of 16 adverse events (Table 11) were reported by 8 participants, with 4 post-emergent AEs defined as potentially related to SPH (constipation, paresthesia, tension headache, and excessive thirst). All AEs were resolved by the study's conclusion. One serious adverse event (SAE) occurred, in which a participant experienced significant symptoms of anxiety, but it was deemed unrelated to SPH. This study participant received treatment and discontinued the supplementation protocol. No deaths or other significant AEs were reported.

Table 10. Adverse Events (AEs) by System Organ Class and Participant Incidence (n = 20).

System Organ Class	Preferred Term	Lower-Level Term	Number of AEs	Participants Experiencing AEs n (%)
Gastrointestinal disorders	Constipation	Constipation	1	1 (5%)
General disorders	Thirst	Excessive thirst	1	1 (5%)
Immune system	Allergic reaction to excipient	Allergic reaction to drug excipient	1	1 (5%)
Infections	Nasopharyngitis	Cold	1	1 (5%)
Musculoskeletal	Back pain, myalgia, pain in extremity	Back, muscle, leg pain	4	2 (10%)
Neoplasms benign, malignant and unspecified (including cysts and polyps)	Synovial cyst	Ganglion	1	1 (5%)
Nervous system disorders	Peripheral neuropathy, paresthesia, tension headache	Neuropathy, paresthesia, tension headache	4	3 (15%)
Psychiatric disorders	Mental disorder	Nervousness	1	1 (5%)
Respiratory, thoracic and disorders	Cough	Cough	1	1 (5%)
Vascular disorders	Migraine	Migraine	1	1 (5%)

DISCUSSION

This proof-of-principle clinical trial provides initial evidence that daily lower-dose supplementation with SPH may offer health benefits, specifically in enhancing vitality, regulating oxidative stress, and modulating inflammatory and hematological markers. These findings at a daily intake of 4 g are generally consistent with the reported effects of higher SPH doses (12.0 g to 16.0 g per day). This suggests that lower doses of 1–2.0 g per day may produce similar outcomes; however, this hypothesis remains untested in clinical trials.

A key finding of this study was the significant enhancement in perceived vitality among participants in the PP population, reflected by a 10.24% relative increase from baseline to the end of the study ($p = 0.004$). This suggests that SPH supplementation can positively impact overall feelings of vitality, which potentially links to improved iron metabolism, as indicated by *FTH1* up regulation in previous SPH investigations [30]. Vitality encompasses an individual's overall physical well-being, including qualities such as resilience and endurance, qualities that enable optimal homeostasis to be

maintained in the presence of daily stressors [36]. The effects were more pronounced in the female group, who exhibited a 14.55% increase in self-assessed vitality ($p = 0.008$), whereas the improvement observed in males did not reach statistical significance ($p = 0.36$). To the extent that iron homeostasis contributed to the observed efficacy, it is conceivable that this gender difference is partly due to the higher iron requirements in women.

Participants also reported significant improvements in specific physical and mental aspects of daily life. Improvements in key physical and mental aspects of daily life were demonstrated by enhanced scores across multiple parameters of the Vitality Quality of Life (VQoL) questionnaire. Participants reported increased feelings of energy and vitality, with significant improvements in overall energy levels ($p = 0.02$) and alertness ($p = 0.03$). These improvements were further corroborated by a reduction in midday slumps ($p = 0.01$) and a greater ability to sustain energy output throughout the day ($p = 0.006$). Additionally, the reduction in difficulty performing vigorous activities and the decreased frequency of lethargy indicate that SPH not only enhances overall energy levels but also supports sustained energy maintenance during daily tasks. In terms of psychological and social well-being, participants experienced significant increases in enthusiasm and engagement in relationships ($p = 0.002$), alongside a marked reduction in irritability ($p < 0.001$). Cognitive benefits were reported, including improved task initiation ($p < 0.001$) and enhanced concentration on tasks ($p = 0.016$), which contribute to greater mental clarity and productivity. While the overall change in perceived sleep quality did not reach statistical significance ($p = 0.11$), there were positive trends in reducing mild symptoms of sleep-onset insomnia, with participants feeling more energized upon waking ($p = 0.006$). Overall, supplementation over 18 weeks appears to positively affect various dimensions of well-being, including physical energy, mental clarity, emotional stability, and social engagement. It may also provide ancillary benefits for sleep-related outcomes.

The hematological findings from this study provide insights into the potential effects of SPH supplementation on erythropoiesis. Among healthy adult subjects, a significant reduction in red cell distribution width (RDW) of 0.8% ($p = 0.004$) was observed, indicating improved homogeneity in red blood cell size [35]. While the mean changes were not numerically substantial, it may suggest a more stable erythropoiesis, which would be beneficial for overall red blood cell function. In the male subgroup, a 5.2% increase in hemoglobin levels ($p = 0.05$) and a significant rise in red blood cell count ($p = 0.04$) were observed, highlighting a tendency toward enhanced erythropoiesis. These findings align with the approximately four-fold increase in *FTH1* expression, which encodes the ferritin heavy chain, a crucial protein for iron storage, iron utilization and overall iron regulation. These results are consistent with prior clinical research showing that daily SPH supplementation significantly increased hemoglobin and serum ferritin levels [30]. Overall, the changes in hematological parameters suggest that a daily dose of 4g of SPH may support iron metabolism and erythropoiesis, as several indicators show trends that reflect a positive effect on these processes.

A key aspect of this study was the up regulation of genes involved in antioxidant and iron metabolism, among them *HMOX1* and *FTH1* which exhibited nearly a four-fold increase in expression, along with a 9% reduction in total ROS/RNS free radical activity ($p < 0.001$), suggests that SPH supplementation may help reduce oxidative stress. The analysis of oxidative stress and inflammatory markers corroborates the potential benefits of a daily 4g dose of SPH, supporting its role in

reducing oxidative stress and modulating inflammatory responses. These findings are consistent with prior in vitro studies where SPH significantly UP-regulated key protective genes like *HMOX1* and *FTH1*, indicating its potential to enhance cellular antioxidant defenses [29]. The antioxidant properties of fish hydrolysates, particularly those derived from salmon and mackerel, are well-documented [37]. These properties are attributed to bioactive peptides that scavenge free radicals, compounds that inhibit lipid peroxidation, and trace minerals such as selenium, sourced from salmon head and frames, acting individually or synergistically. The significant lysine content in SPH (7.0 g/100 g) further enhances antioxidant capacity, while the high levels of proline and arginine contribute to these effects by supporting hydrophobic interactions and nitric oxide production [38]. The observation of these effects in whole blood samples from humans implies that SPH could play a role in mitigating oxidative stress, which is relevant for healthy aging and the prevention of chronic diseases. SPH supplementation also modulated inflammatory markers, showing increases in IL-2 and IL-10 ($p < 0.001$ for both) and decreases in IL-5, IL-8, and CRP ($p < 0.001$ for all). This suggests that SPH promotes an anti-inflammatory profile, contributing to a healthier immune response. These changes align with prior studies showing significant reductions in IL-6 and other metabolic biomarkers, along with a 5.9% decrease in BMI compared to whey protein isolate control, reinforcing SPH's anti-inflammatory potential [31]. The substantial fold-change of *HMOX1* (4.09 ± 0.22), known for its role in anti-inflammatory responses and antioxidant defense mechanisms, further supports this potential. Additionally, IL-10, an anti-inflammatory cytokine that can induce *HMOX1* expression, promotes cytoprotective effects and reduces oxidative stress. The concurrent increase in IL-10 and up regulation of *HMOX1* may represent a pathway through which SPH exerts its beneficial effects on inflammation, oxidative stress, and indirectly through improved gut health [39].

Previous preclinical studies have demonstrated the glucoregulatory properties of SPH, showing that it significantly inhibits DPP-IV activity and enhances glucose uptake in muscle cells, suggesting its potential for managing glucose metabolism [32]. However, the glycemic outcomes observed in our study were modest, which is expected in a healthy population with normal body mass. While there was a statistically significant increase in HbA1c levels (mean change of 0.09%, $p = 0.01$), the reduction in fasting plasma glucose (FPG) levels approached a statistically significant decrease (mean change of -0.19 mmol/L, $p = 0.06$). Subgroup analysis revealed that males experienced a significant increase in HbA1c (mean change of 0.2%; $p = 0.02$) with no significant change in FPG (mean change of -0.1 mmol/L). In contrast, females showed a statistically significant decrease in FPG (mean change of -0.23 mmol/L; $p = 0.05$) without a significant change in HbA1c (mean change of 0.04%; $p = 0.21$). In a previous study, supplementation with 12.0 g of SPH per day for eight weeks resulted in approximately 6% reductions in both FBG and HbA1c, whereas the active comparator, whey protein isolate, showed no effect [40]. Combined with the established bioactive glucoregulatory properties of SPH, these findings highlight the need for further investigation into its effects on glucose metabolism.

Finally, the findings across all analyses show a statistically significant improvement of a magnitude that is likely to be clinically meaningful in self-perceived hair, nail, and skin health improvements, particularly among the responder population ($p = 0.004$) and female participants ($p = 0.009$), with relative improvements of 32.8% and 34.5%, respectively. In contrast, the male subgroup did not exhibit a significant change ($p = 0.25$),

which may reflect the well-documented gender differences in use of beauty-related products. The relatively lower engagement in skincare practices and reduced attention to skin condition and overall appearance may have influenced the self-reported effects on skin quality among men in this study. These findings are consistent with research indicating that marine collagen, especially in hydrolyzed form, can markedly enhance skin elasticity and hydration, thereby diminishing visible signs of aging. Notably, these benefits appear more pronounced in women compared to men [41].

In summary, this study provides initial evidence that SPH supplementation supports physical and psychological energy levels and enhances vitality. Notable improvements were observed in specific hematological parameters, along with beneficial modulation of oxidative stress and inflammatory responses. The changes in gene expression in white blood cells (WBCs) suggest broader systemic benefits, including enhanced total antioxidant defenses. Additionally, a measurable trend towards reduced pro-inflammatory and increased anti-inflammatory signaling, indicated by shifts in cytokines and inflammatory biomarkers, aligns with the observed gene expression modulation, further reinforcing the study's findings.

This study has several limitations. It was conducted with a small sample size and lacked a control group, leaving the potential influence of a placebo effect unknown. Additionally, the absence of a control group makes it challenging to account for natural variations in health over time, which could influence the outcomes observed in this study. The results are, however, consistent with those reported in previous randomized trials of SPH, suggesting that the observed health benefits are likely attributable to the intervention. Moreover, the study did not investigate a dose-response relationship to determine whether higher doses of SPH might provide greater health benefits. Notably, the gene expression modulation observed in this study with a lower daily dose of 4.0 g over a longer duration was comparable to the effects reported with significantly higher doses of 12.0 g to 16.0 g over shorter durations. This suggests that extended supplementation at lower doses may achieve similar benefits to those seen with higher doses in a shorter time frame. One limitation of this study is the reliance of a non-validated Vitality and Quality of Life (VQoL) Questionnaire, which may affect the reliability and generalizability of the self-reported results, as the tool has not undergone formal testing to verify its effectiveness in assessing these measures. The absence of power calculations and the use of a subjective questionnaire highlight the need for careful evaluation of the findings and objective measures of improved beauty metrics should be undertaken. A further limitation is the lack of control for dietary variations, as participants were asked to maintain their usual diets without tracking specific dietary patterns or intolerances, except for those related to the test material's ingredients. While low-protein diets, energy supplements and known allergies to salmon proteins were exclusion criteria, other dietary factors were not systematically controlled. Stricter dietary controls in future studies could minimize potential confounding variables and provide clearer interpretations.

Despite these limitations, the consistency of results across various measures and data sets enhances the credibility and validity of the findings, supporting the potential of SPH supplementation to promote human health at a lower daily dose. Establishing appropriate dosages of bioactive compounds is critical for maximizing efficacy and safety in functional food applications [42]. Larger-scale clinical trials with control groups and objective measures are needed to confirm the health

benefits of SPH and explore its full potential. Future studies should also investigate the molecular mechanisms through which bioactive peptides in SPH exert their effects. It is worth considering whether daily administration of the SPH may have positively influenced sleep, potentially contributing to improved vitality and well-being, as observed in previous randomized controlled trials [44]. Dietary intake of marine hydrolysates is likely to influence the gut microbiome, potentially impacting a wide range of diseases, another area of research that should be further explored. Furthermore, a nutrimetabolomics analysis of salmon identified 508 bioactive compounds, with 143 detected in human plasma post-consumption, some of which were associated with improvements in cardiometabolic health indicators. These findings underscore the potential for fish-derived hydrolysates like SPH to deliver similar benefits through bioactive compounds. Investigating bioactives in SPH, their mechanisms of action, and their interactions with molecular pathways could provide deeper insights into their role in promoting human health, further emphasizing the relevance of functional food research in enhancing well-being [43]. Challenges related to raw material variability and the high costs of peptide isolation and purification must also be appropriately addressed to facilitate its large-scale application as a functional food ingredient [45].

CONCLUSIONS

This pilot study suggests that daily supplementation with 4 grams of salmon protein hydrolysate (SPH) may enhance vitality, reduce oxidative stress, and favorably influence inflammatory markers in healthy adults. Key findings include a significant increase in self-assessed vitality (10.24%, $p = 0.004$), reductions in oxidative stress biomarkers (9%, $p < 0.001$), and up regulation of antioxidant genes such as *HMOX1* and *FTH1*. Inflammatory markers shifted towards an anti-inflammatory profile, with increases in IL-2 and IL-10, and reductions in IL-5, IL-8, and CRP (all $p < 0.001$). Additionally, participants reported improved cosmetic outcomes, particularly among women, with a 23.7% enhancement in hair, skin, and nail quality ($p = 0.03$).

From a practical standpoint, SPH shows promise as a nutraceutical supplement for promoting healthy aging, reducing inflammation, and enhancing skin and cosmetic health. Marine-derived protein hydrolysates, like the one studied, offer a sustainable method to utilize marine by-products, adding economic value while delivering health benefits. By-products such as frames, heads, and skin are rich in bioactive peptides and compounds with clinically significant effects. When properly sourced, marine protein hydrolysates are safe at therapeutic doses and widely accessible. Future research should prioritize well-designed, larger-scale clinical trials tailored to nutritional studies to further validate SPH as a functional food ingredient. Additionally, investigating the effects of these hydrolysates on the intestinal microbiome and its potential role in mediating health benefits is an important avenue for future exploration.

List of Abbreviations: SPH: Salmon Protein Hydrolysate, IL: Interleukin, CRP: C-Reactive Protein, BMI: Body Mass Index, HbA1c: Glycated Hemoglobin, FPG: Fasting Plasma Glucose, RBC: Red Blood Cell, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration, RDW: Red Cell Distribution Width, ROS: Reactive Oxygen Species, RNS: Reactive Nitrogen Species, HMOX1: Heme Oxygenase-1, FTH1: Ferritin Heavy Polypeptide-1, APOE: Apolipoprotein E, GCLC: Glutamate-Cysteine Ligase Catalytic Subunit, GPX1: Glutathione Peroxidase 1, NQO1: NAD(P)H Quinone Dehydrogenase 1, SOD1: Superoxide Dismutase 1, VQoL: Vitality and Quality of

Life, LOCF: Last Observation Carried Forward, SAE: Serious Adverse Event, CRO: Contract Research Organization.

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