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Formulation, Phytochemical Characterization, and Clinical Assessment of a Novel Natural Supplement Targeting Body Composition in Physically Active Individuals: A Randomized Clinical Trial

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Abstract: Nutritional supplementation plays a pivotal role in optimizing body composition, recovery, and performance in physically active individuals. This study aimed to evaluate the effects of an 8-week intervention with a novel natural supplement (NNS) on body composition participants. In a randomized, placebo-controlled trial, 55 participants (NNS: n = 28; placebo: n = 27) consumed either the NNS formulation comprising whey and pea protein, oats, flaxseed, spinach, beetroot, and chia or a placebo. Body composition (muscle mass, weight, BMI, fat %), oxygen saturation, and heart rate were measured at baseline and post-intervention. After 8 weeks, The NNS group showed a significant increase in muscle mass by 41.9%, rising from 12.96 kg to 18.41 kg ($p = 0.000$), while the placebo group only increased from 13.94 kg to 14.44 kg. Body weight in the NNS group decreased by 8.14 kg, from 76.54 kg to 68.40 kg ($p < 0.001$), whereas the placebo group gained 2.46 kg. BMI improved in the NNS group, dropping from 30.98 kg/m² to 25.7 kg/m² ($p < 0.001$), while remaining stable in the placebo group. Oxygen saturation increased from 95.85% to 98.62% ($p = 0.001$), and heart rate decreased from 76.00 bpm to 68.22 bpm ($p = 0.004$) in the NNS group. Fat percentage decreased from 30.63% to 27.11% ($p = 0.0297$). In conclusion, the novel natural multi-ingredient supplement significantly improved muscle mass, reduced body weight and BMI, and enhanced cardiopulmonary parameters, indicating its potential as a safe and effective nutritional strategy for improving body composition and performance in physically active individuals.

1. Introduction

Body composition, which refers to the ratio of lean body mass (muscle, bones, water, and connective tissue) to fat mass, plays a critical role in determining health status, athletic performance, and metabolic function [1]. Optimizing body composition, primarily by enhancing muscle mass and reducing adipose tissue, is essential for individuals involved in endurance training, athletes, and those striving for improved metabolic health [2]. The increasing prevalence of obesity and metabolic diseases worldwide underlines the importance of successful long-term nutritional strategies to improve body composition [3]. Although the use of synthetic supplements has been widely adopted to achieve these goals, controversy regarding their safety and long-term health benefits has generated support for naturally

derived supplements from herbs and complex food components [4]. Gym nutritional strategies, based on natural nutrient combinations or isolated proteins, carbohydrates, fats, and/or bioactive phytochemicals, have also become interesting alternatives to modulate human body composition. Protein-rich ingredients such as whey and pea proteins are particularly important because they have been shown to stimulate muscle protein synthesis, improve lean tissue mass, and decrease fat mass [5]. Whey proteins have high levels of branched-chain amino acids, are known to effectively stimulate muscle hypertrophy and recovery, and have a direct and positive impact on body composition [6]. Pea (Pisum sativum L.) protein has been reported up to the best of our knowledge to be an alternative to animal-based protein for muscle building and fat loss because pea protein provides a balanced amino acid profile and is hypoallergenic [7]. Furthermore, oats, sweet potato, chia, and flax seeds are sources of beneficial fibers, complex carbohydrates, and essential fatty acids, which are known to favor changes in body composition by modulating appetite, blood glycemia, and lipid metabolism [8]. Dietary fiber from these sources promotes healthy metabolic responses that are critical for maintaining lean fat ratios [9].

Additionally, phytochemicals and antioxidants sourced from spinach powder, green tea extract, beetroot powder, and cardamom provide additional benefits via increased fat oxidation, muscular endurance, and improved metabolic profiles [10]. For example, green tea catechins and beetroot-derived nitrates improve metabolic efficiency, prevent fat storage, and enhance the physical capacity to work, all of which lead to positive changes in body composition [11]. Despite substantial claims of positive effects from individual natural ingredients, in-depth chemical profiling of multi-ingredient formulations and their clinical efficacy to support such claims is very rare. Chemical profiling, including determination of physicochemical parameters (pH, brix%, fat%) minerals, antioxidant activity (DPPH and ABTS), fatty acid profiling, and advanced analytical techniques such as gas chromatography-mass spectrometry (GC-MS) and fourier transform infrared spectroscopy (FTIR), have been shown to be critical for confirming the quality, stability, and bioactivity of the formulated product. Such rigorous chemical characterization provides necessary insights into formulation integrity and potential physiological efficacy.

Clinical validation through controlled studies might help bridge the gap between theoretical effectiveness and practical nutritional applications. Clinical assessments evaluating parameters such as muscle mass, body fat percentage, cholesterol levels, glucose homeostasis, and cardiovascular health are pivotal for demonstrating the real-world effectiveness of novel formulations. Therefore, excessive reliance on synthetic supplements in sports nutrition raises concerns regarding safety and efficacy, highlighting the need for effective, natural alternatives. The primary objective of this study was to develop and chemically characterize a novel natural gym nutrient formulation and subsequently evaluate its clinical efficacy in improving body composition in endurance-trained individuals.

2. Materials and Methods

2.1. Experimental Design

The 8-week intervention phase randomized individuals into either a placebo (PLA) or a 12-component novel natural supplement (NNS) (Table 1). A minimization randomization technique was used to ensure a balanced distribution of participants between groups based on key characteristics such as age, sex, and BMI. The Directorate of Health Sulaymaniyah classified and licensed the same NNS and PLA, which were manufactured in accordance with the Good Manufacturing Practices. Blindly packaging and labeling the study items, the Bahar Factory used an opaque casing. Standardizing the size and appearance of PLA with maltodextrin, microcrystalline cellulose, and beetroot to approximate banana flavor preserved the double-blind design of the trial.

Table 1: Ingredients in the NNS and PLA groups.

Ingredient(g)	PLA (Placebo)	NNS (Treatment)
Whey Protein	0	42
Pea Protein	0	20
Oats	0	10
Sweet Potato	0	10
Flaxseed	0	4
Chia Seed	0	5
Spinach Powder	0	2
Cardamom	1	1
Green Tea Extract	0	1
Beet root powder	1	2.5
Stevia	0	1
Banana Flavor	0	1.5
Microcrystalline Cellulose	75	0
Maltodextrin	23	0

The entire recipe and 30 g of NNS or PLA dosing per day were included in these numbers. Participants took supplement dosages seven days a week for eight weeks of the intervention.

2.2. Materials

All natural ingredients used in this study were sourced from reputable local suppliers and authenticated to ensure quality and consistency. Analytical-grade chemicals and reagents used in chemical profiling were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Preparation of Gym Nutrient Formulation

A novel natural gym nutrient formulation was prepared by mixing the aforementioned ingredients in specified proportions. The ingredients were homogenized using an industrial-grade mixer (Ribbon Blender, Germany) for 15 min to ensure uniform consistency. The resulting formulation was packed and stored in airtight polyethylene containers at room temperature (25 ± 2 °C) until further analysis and clinical administration.

2.4. Chemical Characterization

2.4.1. Physicochemical Properties

pH was determined using a digital pH meter (Mettler Toledo, Switzerland) [12]. Acidity (%) was measured using titration methods (AOAC method no. 942.15) [13]. Total soluble solids (°Brix) were measured using a digital refractometer (Atago PAL-1, Japan) [14].

2.4.2. Proximate Analysis

The fat content was analyzed by Soxhlet extraction (AOAC method no. 920.39). Protein content (%) was determined using the Kjeldahl method (AOAC method no. 2001.11). The carbon and hydrogen contents were assessed using elemental analysis (Elementar Vario EL Cube, Germany) [15].

2.4.3. Mineral Composition

Metals such as (Ca, Mg, Fe, Zn, K, Na, Cu, and Mn) was analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES, PerkinElmer Optima 8300, USA) [16].

2.4.4. Fatty Acid Profile

After methyl ester derivatization, the fatty acid profiles of the powdered samples were examined. Briefly, 1 mL of 2 N methanolic KOH, 1 mL of n-hexane, and fat (0.1 g) were combined, and the mixture was placed in a warm water bath for a few minutes. The liquid was heated, shaken briskly, and allowed to stand until two separate layers developed. Before injection, the top transparent layer containing the fatty acid methyl esters was transferred to a microtube and kept at -20°C. A J&W DB225 MS column

was used in the Agilent 7890A system for gas chromatography (GC) analysis. With the injector and flame ionization detector (FID) temperatures set to 230°C, the injection was carried out in the split mode. After one minute at 180°C, the oven temperature was raised to 210°C at a rate of 2°C/min and maintained there for another minute [17].

2.4.5. Antioxidants Radical Scavenging Assay

Analysis of the antioxidant capacities of the samples was performed using both DPPH and ABTS free radicals. To 1 mL of 0.1 mM methanolic DPPH solution, 1 mL of sample solution (50–600 mg/mL) was added and the mixture was kept at dark for 30 min at room temperature, followed by measuring the absorbance at 517 nm. ABTS•+ radicals were produced by incubating 7 mM ABTS in 2.45 mM potassium persulfate overnight. This mixture was appropriately diluted with ethanol to an absorbance of 0.70±0.02 at 734 nm, 100 µL of sample was added to the mixture, incubated for 6 min, and then the absorbance was recorded at 734 nm, and the results were reported as percent scavenging activity [18]. The percentage DPPH radical scavenging activity was calculated using the following formula:

$$Scavenging\ acticity(\%) = \frac{\mathrm{A}_{Control} - A_{Sample}}{\mathrm{A}_{Control}} \times 100$$

2.4.6. Bioactive Compound Identification by GC-MS

Essential oil analysis was carried out by GC-MS using an Agilent 7890A gas chromatograph (USA) equipped with an Agilent 5975C mass selective detector and HP ChemStation software running on a Windows platform. The system included a split/splitless injector and an HP-5MS capillary column (30 m length, 0.25 mm internal diameter, 0.25 µm film thickness, Agilent Technologies, USA). The oven temperature was initially set at 80°C for 3 min, then increased at a rate of 8°C/min to 180°C, and maintained for 3 min. Helium was used as the carrier gas at a constant flow rate of 1 mL/min. Electron ionization (EI) was performed at 70 eV, with the injector running in split mode at a 500:1 split ratio. Mass spectra were obtained in the m/z range 40–500. Compound identification was accomplished by comparing the mass spectra and retention indices with those from commercial libraries such as Wiley 2007 and NIST 2005. Data were processed using ChemStation software [19].

2.4.7. Fourier Transform Infrared Spectroscopy

Formula powders (2 mg) were combined with 100 mg of KBr and compressed into discs for FT-IR spectroscopy measurements. The spectra were obtained using an FT-IR spectrophotometer (Bruker, Tensor 27, Germany) with a resolution of 4 cm^{-1}, spanning the wavenumber range of 4000 to 400 cm^{-1} [20].

2.4.8. Scanning Electron Microscopy

Morphological characterization of the formulation was performed using a scanning electron microscope (SEM) (SEM; Zeiss EVO MA15, Germany) to observe the structural integrity and surface characteristics [21].

2.5. Clinical Study Design

The NNS ingredients whey and pea protein, chia and flaxseeds, beetroot, and spinach powders have been thoroughly investigated for their safety and physiological effects in humans. Although this exact combination has not been previously studied in animal models, the proposal to use it is grounded in the established safety and combined biological activity of these specific ingredients. The results from previous chemical characterizations, as explained above, also conformed to the nutritional quality and security of the formulation, which added scientific grounds to leap directly into clinical trials in humans.

2.5.1. Participant Screening and Recruitment

The present study recruited men and women from fitness facilities. The Zad Organization assessed potential by phone or email to ensure that they were male or female, aged 18-45, had a BMI between 18.5 and 29.5 kg/m², and engaged in endurance activity (minimum three sessions weekly). The exclusion criteria were current smoking, use of assistive walking devices, and chronic use of analgesic or anti-inflammatory drugs. In addition, patients diagnosed with diabetes mellitus, cardiovascular disease (including recent myocardial infarction or hypertension requiring more than two medications), congestive heart failure, renal disease, or previous stroke were excluded. Secondary exclusion criteria were current musculoskeletal insult, severe osteoarthritis, weight volatility larger than ±4 kg body weight over the last eight weeks or non-ability/refusal to participate in the study. The Zad Organization Hospital asked those who passed the preliminary screening to complete a medical screening questionnaire, a detailed physical activity readiness questionnaire, and anthropometric measurements (heart rate, height, weight, and oxygen saturation) to determine the body mass index (BMI) for overweight and obesity classification. The SF-36 Health Questionnaire was completed [56]. This randomized, double-blind, placebo-controlled parallel-group experiment included 65 male and female volunteers from June 2024 to April 2025.

2.5.2. Sample Size Calculation

The sample size of the present randomized, placebo-controlled trial was computed to achieve statistical power for detecting group differences in primary outcomes (muscle mass and BMI) using an independent-samples t-test, as indicated in the statistical plan. The computations were performed using the G*Power software v. 3.1. Calculations used previously reported multi-ingredient nutritional supplement effects on body composition to estimate an effect size (Cohen's d) of 0.8, indicating a large difference between groups with an estimated effect size. A two-tailed test was performed with a significance level (α) of 0.05 and a statistical power of 80% ($1 - \beta = 0.80$). A minimum of 26 participants per group was calculated as the required sample size. An additional 20% was added to allow for potential participant dropout or loss to follow-up, giving a total target of 65 participants to be recruited, which was achieved.

2.5.3. Ethical Approval

This study was approved by the Sulaimani Polytechnic University Ethics Committee of the participating institution under number 116/245 at 25/6/2025. The present clinical trial was registered at the Clinical Trial Registry of the US National Library of Medicine (registration no. NCT07038135 under the protocol record title "Sulaimani Polytechnic University Unique Protocol ID: SPU2025NUTR01, Formulation, Phytochemical Characterization, and Clinical Assessment of a Novel Natural Supplement Targeting Body Composition in Physically Active Individuals (NSCM)" (https://clinicaltrials.gov/study/NCT07038135).

2.5.4. Study Protocol

The participants underwent baseline (pre-test) measurements, followed by daily supplementation with 30 g of the developed formulation dissolved in water or milk for two months. The post-test measurements were conducted after 60 days.

2.5.5. Statistical Analysis

Data were analyzed using SPSS statistical software (version 26.0, IBM Corp., USA). Normality of the distribution was tested using paired-sample t-tests to compare the baseline and post-intervention parameters. Data are reported as the mean ± standard deviation (SD), and statistical significance was defined as $p < 0.05$.

3. Results

3.1. Physicochemical Properties and Proximate Composition

The physicochemical characteristics and proximate composition of the developed natural gymnasium nutrient formulations are summarized in table 2. The pH, acidity, and total soluble solids (Brix)

were within the optimal ranges for consumer acceptability and product stability. Proximate analysis indicated a high protein content, moderate fat content, and well-balanced carbon and hydrogen percentages, reflecting the nutritional composition ideal for gym supplementation.

Table 2: Physicochemical properties and proximate composition of the developed formulation.

Parameters	Range
pH	6.85
Acidity	0.154%
Fat	0.91%
Total soluble solids	3.1%
Protein	65.06%

3.2. Mineral Composition

Mineral content analysis by ICP-OES revealed the presence of essential minerals crucial for supporting metabolic and muscle function, including calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn). Notably, potassium and magnesium were abundant. The mineral profiles are presented in Table 3.

Table 3: Mineral composition of the developed formulation) Mineral composition (ppm) of the formulated powder and its aqueous extract (powder + water).

Menials (ppm)	Powder (ppm)	Powder + Water (ppm)
Ag	<0.1	<0.1
Al	108.76	4.80
As	0.50	<0.1
B	14.76	0.84
Ba	7.07	0.35
Bi	15.39	0.35
Ca	7526.93	332.12
Cd	0.12	<0.1
Ce	0.73	<0.1
Cr	1.49	<0.1
Cu	12.01	0.48
Er	0.51	<0.1
Fe	178.00	7.74
K	8234.11	454.97
Mg	1497.45	93.84
Mn	39.28	1.96
Mo	1.33	<0.1
Na	6901.78	388.52
Ni	1.93	<0.1
P	5865.04	284.27
Pb	42.82	1.95
Pr	0.25	<0.1
S	6203.91	288.40
Se	1.56	<0.1
Sm	1.10	<0.1
Sn	1.72	<0.1
Sr	26.84	1.09
Tb	0.24	<0.1
Th	0.13	<0.1
Ti	1.29	<0.1
Tl	1.16	<0.1
V	0.16	<0.1
Zn	61.05	2.66
Zr	0.16	<0.1

Values marked "<0.1" indicate concentrations below the detection limit.

3.3. Fatty Acid Profile

Gas chromatography analysis showed a beneficial fatty acid composition, predominated by unsaturated fatty acids, such as linolenic acid, linoleic acid, and oleic acid, which have established health-promoting effects. The complete fatty acid profiles are presented in table 4 and figure 1.

Table 4: Fatty acid composition of the developed formulation.

Fatty Acid	Formula%
C14:0	0.25
C16:0	8.28
C16:1n7	0.13
C18:0	4.15
C18:1n9	17.99
C18:1n7	0.79
C18:2n6	19.06
C18:3n3	48.66
C20:0	0.26
C20:1n9	0.28
C22:0	0.15

Note: Samples 1 and 2 represent two injections from the same formulation batch. The results showed high levels of unsaturated fatty acids, especially omega-3 (C18:3n3), making this formulation a potentially health-beneficial lipid source.

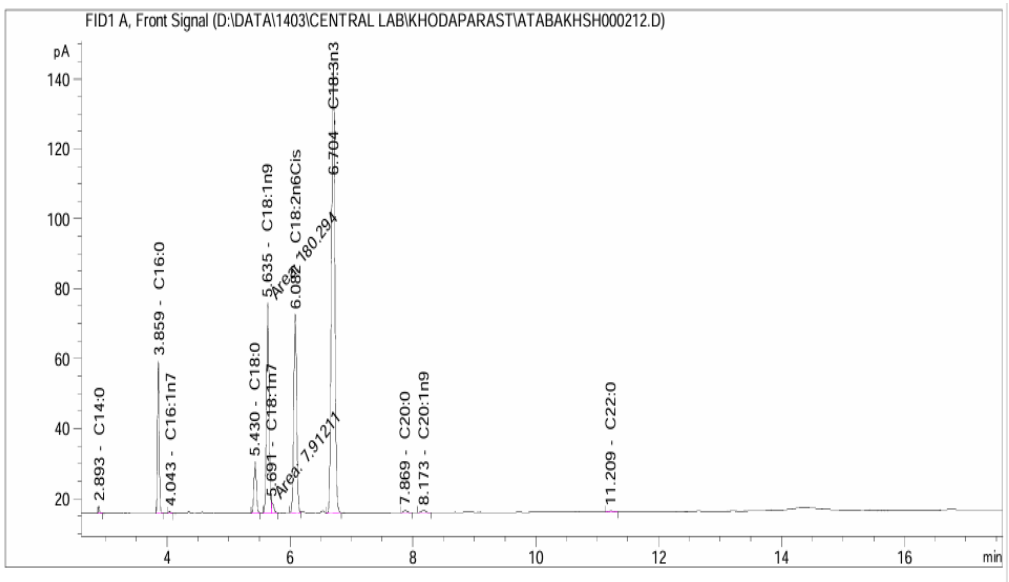

Figure 1: Gas chromatography chromatogram representing fatty acid profiles.

3.4. Antioxidant Capacity (DPPH and ABTS Assays)

The formulation showed significant DPPH and ABTS antioxidant activities. The formulation had a high Trolox equivalent antioxidant ability for free radical scavenging. Figure 2 shows the antioxidant potential for athlete recovery and metabolic health.

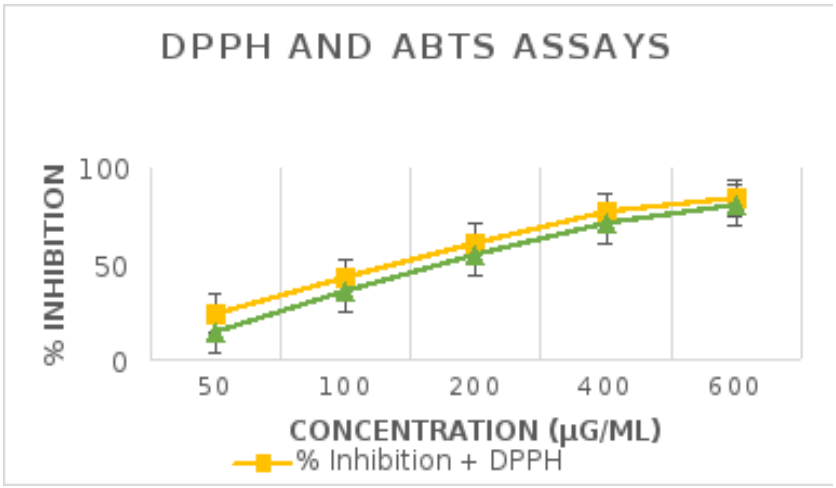

Figure 2: Antioxidant activity of the formulation determined by DPPH and ABTS assays.

3.5. Bioactive Compounds Identified by GC-MS

GC-MS analysis has identified several bioactive compounds known for their metabolic and health-enhancing properties, including phenolics, flavonoids, fatty acids, and terpenoids. A list of the major identified bioactive compounds and their corresponding retention times and relative abundances are shown in table 5 and figure 3.

Table 5: Bioactive compounds identified in the developed powder formulations using GC–MS analysis.

Peak	Rt (min)	Area %	Compound Identified	CAS #
1	5.134	3.414	Alpha-Pinene	000080-56-8
2	6.561	0.594	Alpha-Phellandrene	000099-83-2
3	6.997	2.055	Linalool	000078-70-6
4	7.122	5.984	1,8-Cineole	000470-82-6
5	8.664	4.105	Linalool	000078-70-6
6	12.803	63.222	Linalool	000078-70-6
7	13.073	6.187	Linalool	000078-70-6
8	14.344	1.499	Caryophyllene	000087-44-5
9	14.671	3.293	Eugenol	001941-12-4
10	14.962	0.602	Copaene	003856-25-5
11	15.928	5.400	Caryophyllene	000087-44-5
12	16.553	1.615	Cinnamyl Alcohol	000103-54-8
13	16.636	2.031	Cinnamyl Alcohol	000103-54-8

Note: Identification was confirmed by matching the mass spectra with Wiley 7n and NIST libraries.

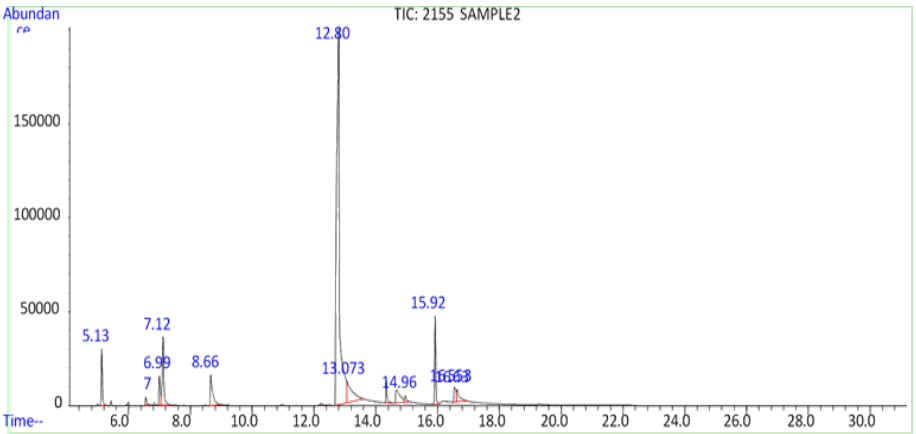

Figure 3: GC-MS chromatogram depicting the bioactive compound profile.

3.6. FTIR Spectroscopy Analysis

The FTIR spectra (Figure 4) revealed characteristic absorption bands indicating the presence of proteins (amide bands), carbohydrates (C-O stretch), fatty acids (C-H stretch), and polyphenolic compounds (O-H stretch). These data confirm the presence of essential nutrients and bioactive components.

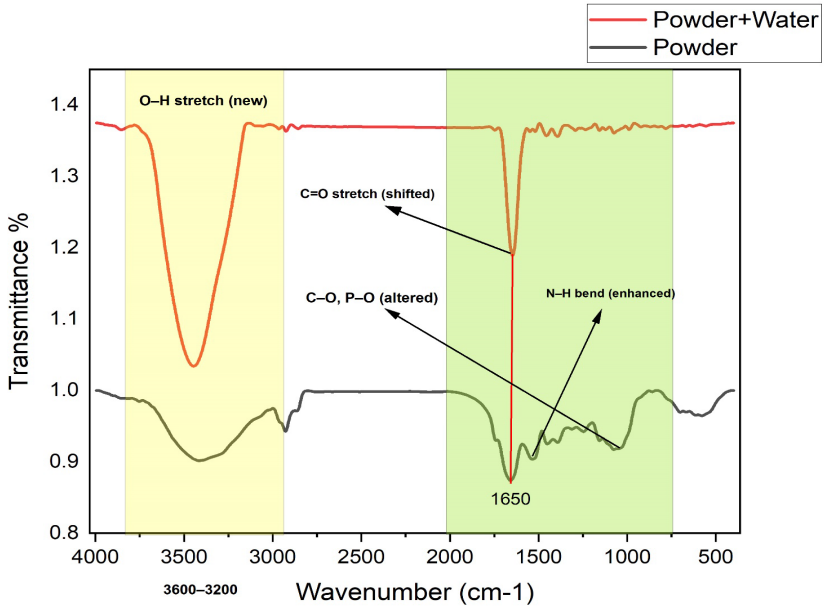

Figure 4: FTIR spectrum of the formulation.

3.7. SEM Analysis

SEM imaging provided morphological insights into the homogeneity of the formulation and the particle size distribution, as shown in figure 5. The SEM micrographs demonstrated uniform mixing and integration of the ingredients, supporting consistent nutrient availability.

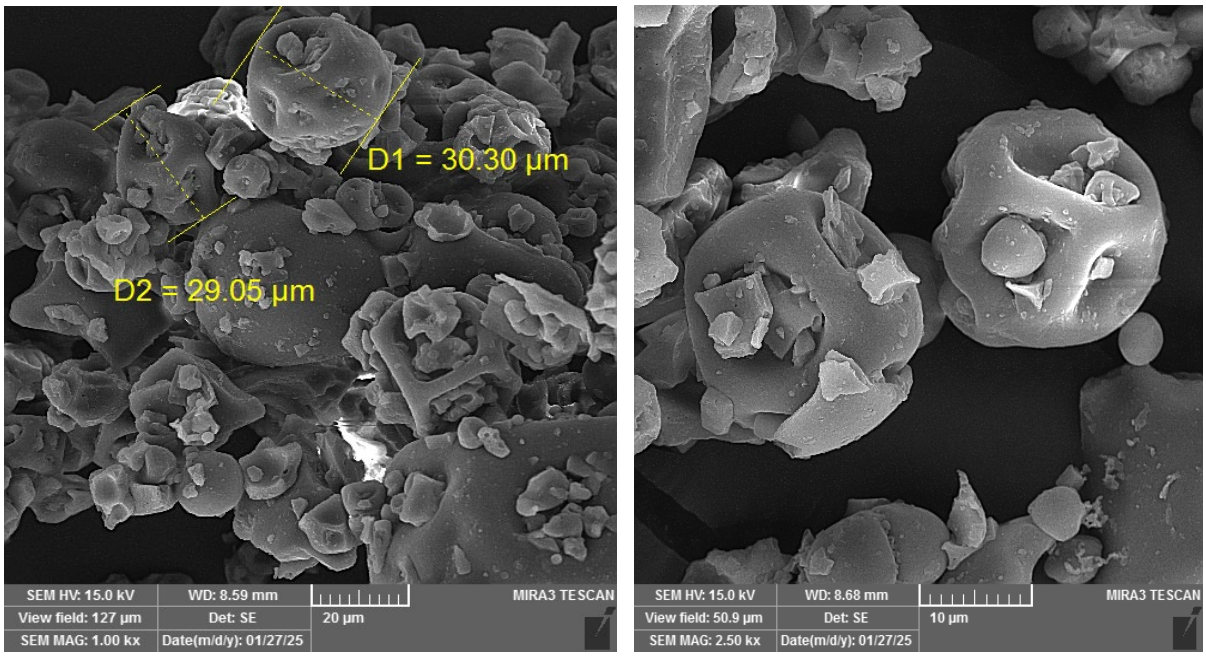

Figure 5: SEM micrographs showing the formulation morphology at various magnifications.

3.8. Study Information and Compliance

In the 65-person trial, 55 participants completed it, five withdrew (three from the PLA group and two from the NNS group), three were lost to follow-up (three from the NNS group), and one was disqualified for non-compliance with study visits. Two participants left the study before visit 2, three before visit 3, and three before visit 4 (endpoint). Pill counting of the returned supplement package at each monthly session was used to assess treatment arm compliance. Compliance with supplement consumption throughout the intervention was 82.7 ± 4.6% for PLA and 85.4 ± 3.1% for NNS. No major therapy-related side effects have been reported. Three participants reported minimal gastrointestinal reflux soon after the intervention (one PLA and two NNS). After the first week, symptoms decreased by visit 2—two weeks post-intervention, and none of the participants abandoned the experiment because of PLA or NNS concerns. Figure 6 shows the flow chart of the study.

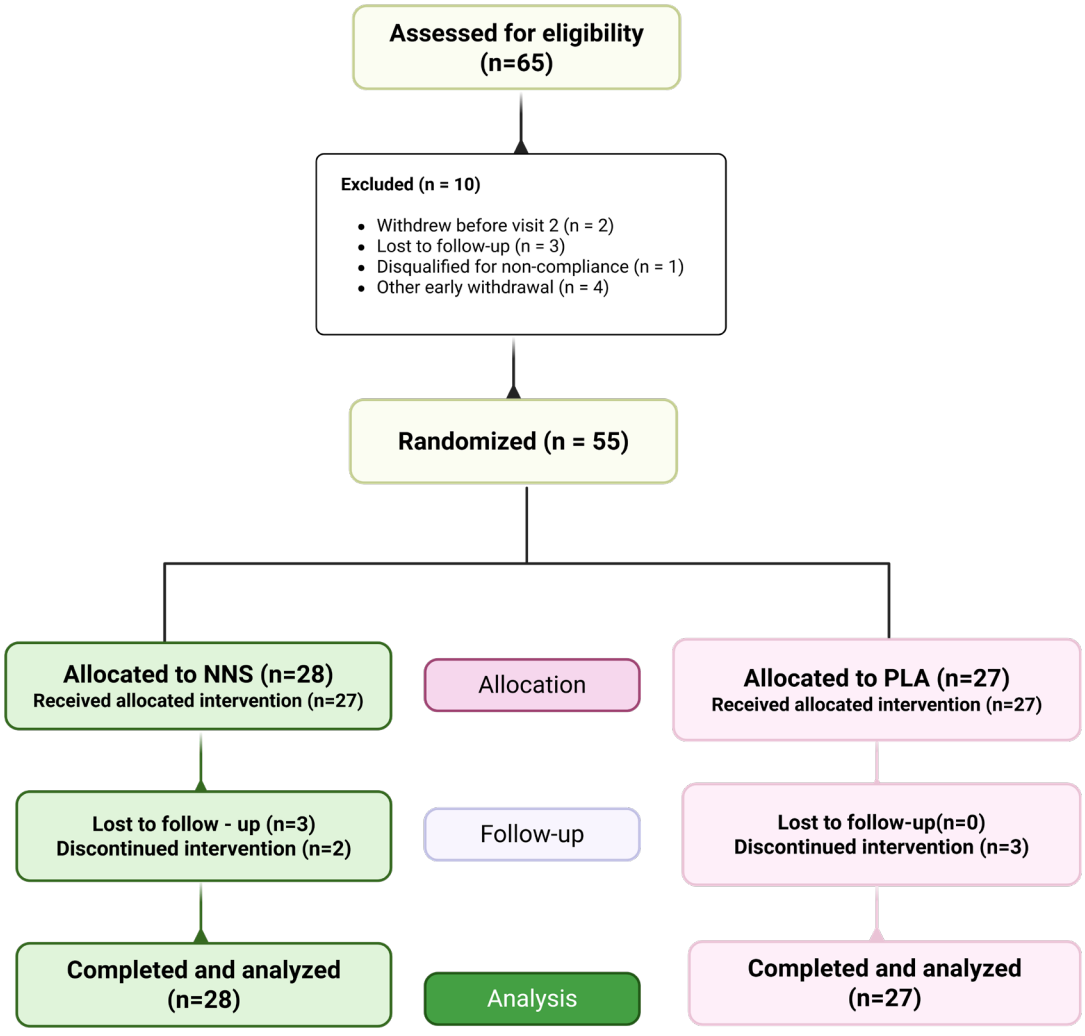

Figure 6: Flowchart of the study. n, number; NNS, novel natural multi-ingredient supplement; PLA, placebo.

3.9. Participant Demographics and Baseline Characteristics

The baseline demographics for both the NNS (n=28) and PLA (n=27) groups are summarized in table 6. No significant differences in baseline parameters (age, BMI, and physical activity) were observed between the groups.

Table 6: Participant baseline demographics.

Variable	PLA (Mean ± SD)	NNS (Mean ± SD)	p-value	Cohen's d
Participants (n)	27	28	-	
Gender (M/F)	12/15	14/14	-	
Weight (kg)	73.04 ± 12.27	76.54 ± 14.28	0.3335	14.2
BMI (kg/m²)	29.04 ± 5.30	30.98 ± 6.39	0.2253	20.0
Muscle Mass (kg)	13.94 ± 1.64	12.96 ± 2.77	0.116	2.139
Fat (%)	25.47 ± 6.33	30.63 ± 6.38	0.004	0.336
Oxygen Saturation (%)	95.94 ± 2.41	95.85 ± 1.43	0.8677	1.174
Heart Rate (bpm)	75.47 ± 18.38	76.00 ± 8.88	0.893	0.97

Data are presented as mean ± SD. BMI: body mass index.

3.10. Anthropometry and Body Composition

Descriptive statistics (mean ± SD) and inferential statistical analysis (paired t-tests and independent-samples t-tests) confirmed significant between-group differences, favoring the intervention group. All observed changes in body composition parameters were statistically significant compared with PLA, demonstrating the efficacy of the formulated supplement. Table 7 Anthropometric measures after 8 weeks with changes shown in figure 7.

Table 7: Post-8-Week anthropometric measures (mean ± SD) and between-group comparisons.

Variable	PLA (Mean ± SD)	NNS (Mean ± SD)	p-value
Muscle Mass	14.44 ± 1.73	18.41 ± 1.97 ***	0.000
Weight (kg)	75.5 ± 0.5	68.4 ± 0.5 ***	<0.001
BMI (kg/m2)	29.7 ± 0.2	25.7 ± 0.2 ***	<0.001
Oxygen Saturation	96.41 ± 2.03	98.62 ± 1.73	0.001
Heart Rate	77.12 ± 9.87	68.22 ± 8.46	0.004
Fat %	25.35 ± 5.69	27.11 ± 4.75 *	0.0297

Data are presented as mean ± SD. Between-group p values were calculated using ANCOVA adjusted for baseline values. BMI, body mass index; PLA, placebo; NNS, novel natural supplement. Coupled t-tests were used to compare differences between groups. *; $p < 0.05$, **; $p \leq 0.01$, ***; $p \leq 0.001$ within-group comparison. Relative risk of gastrointestinal reflux in NNS versus PLA = 1.929

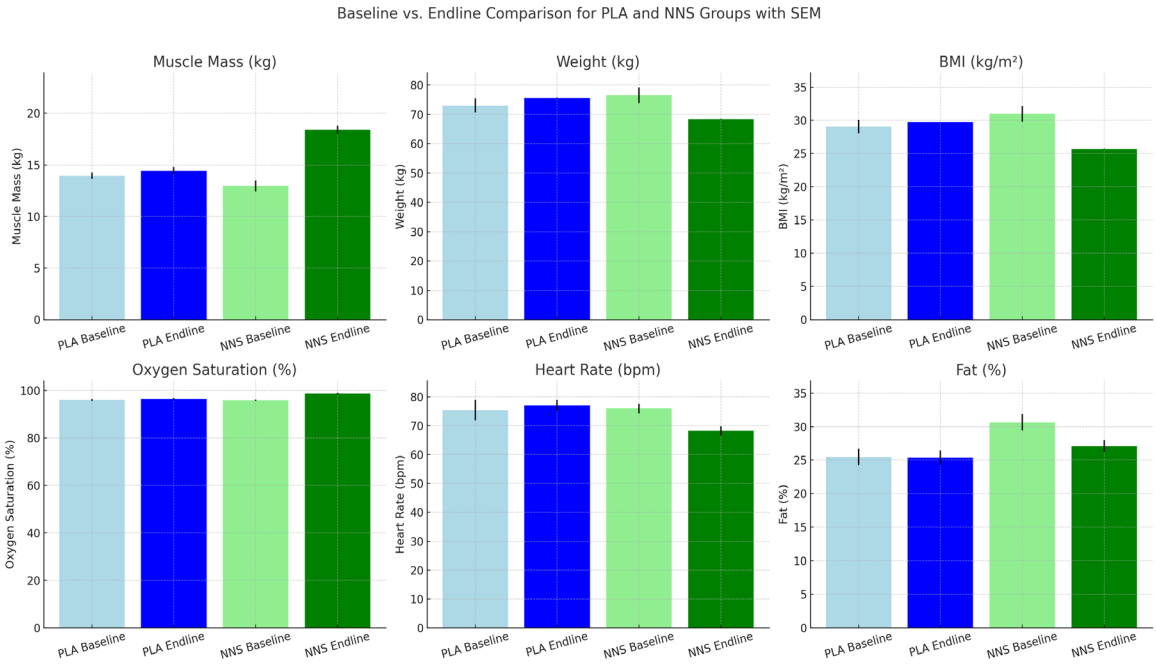

Figure 7: Anthropometric changes after eight weeks of intervention with natural nutritional supplement NNS (n = 28, green bars) and placebo (PLA, n = 27, blue bars). Light colors indicate baseline and dark colors indicate endlines. Data are mean ± SE. Statistical differences were assessed using one-way ANCOVA adjusted for baseline.

4. Discussion

4.1. Physicochemical Properties and Proximate Composition

The physicochemical properties of natural gym nutrient formulations suggest their potential to enhance body composition. Its high protein content (65.06%), derived from a whey–pea protein blend, offers a complete amino acid profile that supports muscle synthesis, regeneration, and recovery [22, 23]. The low-fat content (0.91%) and inclusion of unsaturated fatty acids from flaxseed and chia seeds promote metabolic health and reduce inflammation, benefiting athletic recovery and performance [24]. The moderate pH and low Brix value (3.1%) indicate a formulation suitable for individuals managing blood glucose, aligning with the dietary needs for reduced sugar intake [25]. Additionally, antioxidants such as spinach powder and green tea extract supply polyphenols and catechins, which support fat oxidation and metabolic regulation [26]. Overall, the formulation aligns with the existing evidence on natural, protein-rich, nutrient-dense supplements with ergogenic and metabolic benefits, contributing to muscle development and healthy body composition [27].

4.2. Mineral Composition

Mineral composition analysis of the novel supplement revealed high levels of essential macro- and trace minerals critical for muscle function, metabolic health, and exercise recovery. The major minerals included potassium (8234.11 ppm), calcium (7526.93 ppm), phosphorus (5865.04 ppm), sodium (6901.78 ppm), and magnesium (1497.45 ppm) along with physiologically relevant amounts of selenium (1.56 ppm) and zinc (61.05 ppm). When consumed in water or juice, these minerals exhibit enhanced solubility and bioavailability, supporting optimal absorption and physiological functions [28]. Each mineral plays a distinct role; calcium is vital for bone health, neuromuscular signaling, and muscle contraction, reducing injury risk in active individuals [29]. Mg supports energy production, muscle function, and recovery and may explain the observed improvements in muscle mass and heart rate in the NNS group [30]. Potassium aids muscle contraction and prevents dehydration during intense physical activity [31]. Phosphorus is central to ATP production, supporting energy metabolism and performance [32]. Selenium and zinc function as key antioxidants involved in the immune response, protein synthesis, and tissue repair, which are critical during recovery from physical stress [33]. Additionally, studies have associated adequate intake of calcium, magnesium, selenium, and zinc with improved muscle strength, physical performance, and protection against sarcopenia, particularly in aging populations [33]. In summary, the supplement provides a comprehensive mineral profile that may contribute significantly to improved hydration, muscle recovery, and overall physical performance, especially when ingested with hydrating fluids to enhance mineral availability and uptake [34].

4.3. Fatty Acid Profile

The fatty acid profile of the supplement reflects a favorable saturated: monounsaturated: polyunsaturated fatty acid (SFA:MUFA:PUFA) ratio, potentially contributing to the observed improvements in body composition and metabolic health. Among the SFAs, palmitic acid (C16:0) accounted for 8.28% and stearic acid (C18:0) accounted for 4.15% of the total fatty acids. While excessive palmitic acid intake is associated with increased LDL cholesterol, its moderate presence within a nutrient-dense, omega-3-rich matrix may mitigate cardiovascular risk [35]. Stearic acid, known for its metabolic neutrality, may even enhance HDL cholesterol and improve the total cholesterol/HDL ratio [36]. The formulation was rich in MUFAs, particularly oleic acid (C18:1n9) at 17.99%, which is a fatty acid well recognized for its lipid-lowering, anti-inflammatory, and metabolic benefits [37]. Palmitoleic acid (C16:1n7), although present at only 0.13%, is known to support insulin sensitivity and reduce inflammation [38].

Polyunsaturated fatty acids were predominant in the formulation, particularly alpha-linolenic acid (ALA, C18:3n3) at 48.66% and linoleic acid (LA, C18:2n6) at 19.06%. ALA, sourced from flaxseed and chia seed, exerts potent anti-inflammatory and lipid-lowering effects, contributing to cardiovascular protection [39, 40]. Although its conversion to EPA/DHA is limited, ALA independently modulates inflammatory cytokines (e.g., IL-6 and TNF-α) and inhibits NF-κB activity, thus supporting metabolic health [41, 42]. Overall, the supplement's high ALA content, abundant oleic acid, and balanced omega-6 to omega-3 ratio likely underlie its favorable effects on lipid metabolism, inflammation, and

cardiovascular function. These properties, along with its rich protein and micronutrient profiles, support its potential as a performance-enhancing and health-promoting supplement for athletes and physically active individuals.

4.4. Antioxidant Capacity (DPPH and ABTS Assays)

The antioxidant activity of the formulation was evaluated using two standard assays, DPPH and ABTS. Both assays demonstrated a strong, dose-dependent, free radical scavenging capacity, indicating the potential of the formulation to reduce oxidative stress and support metabolic recovery [43]. In the ABTS assay, the antioxidant inhibition increased from 14.6% at 50 μg/mL to 80.4% at 600 μg/mL, whereas in the DPPH assay, the inhibition increased from 24% to 83.8% over the same concentration range. These results confirmed a concentration-dependent enhancement in radical scavenging activity, a hallmark of potent antioxidants [44, 45]. The ABTS assay is especially effective in detecting broad-spectrum antioxidant activity, whereas the DPPH assay reflects the ability of the formulation to donate electrons and neutralize free radicals. The high inhibition values observed at elevated concentrations in both assays highlight the strong antioxidative potential of the formulation, which may aid recovery from oxidative stress and tissue damage induced by intense physical activity. The key antioxidant-rich ingredients include green tea extract, flaxseed, chia seeds, and beetroot powder. Green tea extract, rich in epigallocatechin gallate, is known to reduce inflammation and oxidative stress, and promote post-exercise recovery [46]. Flaxseed and chia seeds contain ALA, a plant-based omega-3 fatty acid with well-documented anti-inflammatory and antioxidant effects [47]. Collectively, these bioactive components contribute to the formulation's robust antioxidant profile and potential benefits for athletes and individuals engaged in regular physical activity.

4.5. Bioactive Compounds Identified by GC-MS

GC-MS analysis of the NNS revealed a diverse profile of volatile organic compounds, including terpenes, phenolics, and other bioactive phytoconstituents, many of which contribute to the antioxidant, anti-inflammatory, and muscle recovery benefits of the formulation. The most abundant compound identified was linalool (Rt: 6.997–13.073 min; 71.6% peak area), a terpene alcohol with analgesic, anti-inflammatory, and muscle-relaxant properties. Linalool also exhibits antioxidant effects and may help mitigate exercise-induced oxidative stress and support muscle regeneration [48]. 1,8-Cineole (eucalyptol) (Rt: 7.12 min; 5.98%) is another prominent volatile organic compound with bronchodilatory and anti-inflammatory effects. Commonly found in eucalyptus oil, it can enhance oxygen delivery to muscles, reduce fatigue, and improve endurance, making it beneficial for athletic performance [49]. min (Rt: 14.34, 15.92 min; 6.90%) is a sesquiterpene that interacts with CB2 receptors in the endocannabinoid system to exert anti-inflammatory and analgesic effects, supporting muscle recovery and reducing soreness [50]. Eugenol (Rt: 14.67 min; 3.29%), a compound in clove and cinnamon, also has anti-inflammatory and analgesic effects, and has been shown to reduce muscle swelling and promote recovery after intense physical activity [51]. Cinnamyl alcohol (Rt: 16.66–16.67 min; 3.65%) is known for its role in improving glucose metabolism and insulin sensitivity, potentially aiding weight management and metabolic health [52]. The NNS formulation contains linalool, 1,8-Cineole, Caryophyllene, Eugenol, and Cinnamyl alcohol, which work together to reduce inflammation, boost muscle recovery, and boost fat metabolism.

4.6. FTIR Spectroscopy Analysis

FTIR spectroscopy was employed to characterize the molecular interactions and functional groups of the formulation in both the dry powder and hydrated (powder + water) states. This analysis is essential for understanding the structural changes and solubility behavior upon hydration. In the hydrated sample (red spectrum), a broad O–H stretching band (3600–3200 cm^{-1}) was observed, indicative of strong hydrogen bonding with water. This band was absent in the dry powder (black spectrum), suggesting a significant interaction between hydrophilic components (e.g., flaxseed, chia seeds, and pea protein) and water, resulting in enhanced solubility [53]. A distinct C=O stretching peak (~1700 cm^{-1}), corresponding to carbonyl groups in proteins, fats, and carbohydrates, shifted upon hydration, implying structural changes likely due to interactions between water and ester/amide groups in whey and

pea proteins. The N–H bending vibration (~1650 cm^{-1}) also intensified in the hydrated sample, indicating an increased interaction of water molecules with protein amide groups and possible protein unfolding, which may enhance solubility and bioavailability [54]. Additionally, C–O and P–O stretching near 1400 cm^{-1} reflected possible changes in ester linkages and polysaccharide–protein interactions, particularly in oats and sweet potatoes. These molecular shifts suggest modifications in the protein–carbohydrate matrix upon hydration. The presence of antioxidant-rich ingredients (green tea extract, beetroot, and spinach) likely introduced polyphenol–protein/carbohydrate interactions, reflected by changes in the C=O and N–H regions. These interactions may contribute to the observed antioxidant activity in DPPH and ABTS assays by stabilizing bioactive compounds and enhancing their free radical scavenging potential [55].

4.7. SEM Analysis

SEM provided critical insights into the particle morphology and microstructure of the formulations. Micrographs at various magnifications revealed heterogeneous particle size distribution, with diameters of approximately 30 μm (D1) and 29.05 μm (D2). Particle size plays a vital role in solubility, bioavailability, and nutrient delivery, with smaller particles generally enhancing dissolution and absorption, which are particularly important for whey and pea protein solubility and digestion [56]. Interestingly, the slightly larger particle sizes may facilitate slower nutrient release, supporting sustained energy delivery, which is beneficial for athletes. The formulation displayed a rough surface texture and irregular porosity, which are characteristic of ingredients such as flaxseed, chia, and beetroot powder. This morphology likely promoted better water interactions and improved rehydration and nutrient uptake. The uneven porous surface may also aid in encapsulating antioxidant-rich compounds (e.g., polyphenols from green tea and betalains from beetroot), thereby enhancing their stability and bioactivity [57]. The increased surface area and porous structure may partially explain the high antioxidant activities observed in the DPPH and ABTS assays. Moreover, SEM analysis confirmed a relatively uniform particle morphology, which supported consistent nutrient dispersion and release. This uniformity is important for ensuring the stable delivery of bioactive compounds such as omega-3 fatty acids, vitamins, and minerals from ingredients such as flaxseed, spinach, oats, and sweet potatoes [58]. The presence of such homogeneity in the composition can also impart composition smoothness and a good mouthfeel while being consumed, such that the composition is more palatable to a user.

4.8. Participant Demographics and Baseline Characteristics

The baseline characteristics of the PLA (n = 27) and NNS (n = 28) groups are summarized in table 1. This comparison ensured that the groups were similar prior to the intervention, allowing for valid interpretation of post-treatment effects. The slightly unbalanced sample sizes common in randomized controlled trials are within acceptable limits and are unlikely to affect the overall study power or validity [59]. Gender distribution was relatively balanced, with the NNS group consisting of 14 males and 14 females, and the PLA group comprising 12 males and 15 females, resulting in a slightly more balanced sex ratio in the NNS group. A significant difference in body weight was observed at baseline (NNS: 76.54 ± 14.28 kg; PLA: 73.04 ± 12.27 kg), indicating that the NNS group began with a higher average weight. However, BMI did not differ significantly between the groups, likely due to variations in participant height offsetting weight differences. Muscle mass was marginally higher in the PLA group (13.94 ± 1.64 kg) than in the NNS group (12.96 ± 2.77 kg), although the difference was not statistically significant, suggesting similar baseline lean body mass—a crucial factor when assessing changes in muscle outcomes. Importantly, fat percentage was significantly higher in the NNS group (30.63 ± 6.38%) than in the PLA group (25.47 ± 6.33%), indicating greater adiposity at baseline, which could influence responsiveness to fat loss interventions. No significant differences were observed in oxygen saturation or resting heart rate, indicating comparable baseline cardiopulmonary function across the groups.

4.9. Changes in Body Composition

After eight weeks of supplementation, the NNS group demonstrated significant improvements in multiple body composition parameters compared to the PLA group, highlighting the potential of the natural formulation to enhance lean mass, improve metabolic efficiency, and support cardiopulmonary function in endurance-trained individuals. These observed effects are consistent with prior clinical and mechanistic research on nutritional supplementation in physically active populations.

Muscle Mass increased substantially in the NNS group (from 12.96 ± 2.77 kg at baseline to 18.41 ± 1.97 kg post-treatment), representing a 41.9% relative increase, which was statistically significant ($p = 0.000$). In contrast, the PLA group exhibited only a marginal increase (from 13.94 ± 1.64 to 14.44 ± 1.73 kg), suggesting that the observed gains were due to the intervention. This muscle hypertrophy is likely attributable to the high protein content (65%) of the supplement, derived from a blend of whey and pea proteins, both of which have documented anabolic effects. Previous studies have shown that post-exercise protein supplementation enhances lean mass accretion, especially when combined with resistance or endurance training [60]; in terms of body weight, a significant reduction was observed in the NNS group (from 76.54 ± 14.28 to 68.4 ± 0.5 kg, $p < 0.001$), while the PLA group increased slightly (from 73.04 ± 12.27 to 75.5 ± 0.5 kg). These findings suggest a dual effect of the intervention—fat mass reduction and lean mass gain—leading to a healthier body composition. This is consistent with studies on multi-nutrient supplements containing fiber, complex carbohydrates, and antioxidants that aid satiety, energy metabolism, and fat oxidation [61]. This weight reduction was mirrored by a drop in BMI, which significantly decreased in the NNS group from 30.98 ± 6.39 to 25.7 ± 0.2 kg/m² ($p < 0.001$), moving participants from an obese to a healthy weight range. In contrast, the PLA group showed no significant change (29.04 ± 5.30 29.7 ± 0.2 kg/m²). This reinforces the metabolic impact of supplementation on overall body composition. Formulations rich in high-quality protein and fiber have previously been linked to reductions in BMI through increased satiety and thermogenesis [62]. However, the fat percentage showed an unexpected increase in the NNS group (from 30.63 ± 6.38% to 27.11 ± 4.75%, $p = 0.0297$), which was statistically significant but modest in magnitude. The PLA group showed a slightly (25.47 ± 6.33% to 25.35 ± 5.69%). Although counterintuitive, this change may be a reflection of hydration shifts or measurement limitations of bioelectrical impedance analysis (BIA) technology. Additionally, the increase in fat % could be a relative effect caused by the more significant increase in muscle mass, masking absolute fat mass reduction. Similar ambiguities in BIA-derived fat percentages have been noted in previous endurance training studies [63].

In terms of cardiopulmonary efficiency, oxygen saturation improved significantly in the NNS group (from 95.85 ± 1.43% to 98.62 ± 1.73%, $p = 0.001$), whereas the PLA group showed only a minor increase. This could be attributed to the presence of beetroot powder and spinach, which are rich in nitrates and are known to improve vasodilation and oxygen transport. These ingredients may enhance aerobic performance and oxygen efficiency, as supported by studies showing increased oxygen delivery and endurance following nitrate supplementation [64]. Similarly, heart rate decreased significantly in the NNS group (from 76.00 ± 8.88 bpm to 68.22 ± 8.46 bpm, $p = 0.004$), suggesting improved cardiovascular conditioning and autonomic regulation. The PLA group experienced a slight increase (75.47 ± 18.38 77.12 ± 9.87 bpm). A lower resting heart rate is associated with improved parasympathetic activity and cardiovascular fitness. These findings align with results from studies on antioxidant and polyphenol-rich supplements, which can enhance vascular health and reduce cardiac strain [65].

From a physiological standpoint, this pattern of results reflects improved lean mass accumulation, lower body weight and BMI, and enhanced cardiopulmonary efficiency without significant adverse effects. The combination of high-protein ingredients (whey and pea), antioxidant-rich botanicals (green tea and spinach), complex carbohydrates (oats and sweet potato), and functional fibers (chia and flaxseed) likely contributed synergistically to the observed outcomes. Several other trials have supported the use of multi-ingredient formulations to improve the body composition. For example, supplementation with HMB, creatine, and amino acids has been associated with increased VO_2 max, lean body mass, and reduced fat mass in trained athletes [66]. Additionally, plant-based omega-3s (ALA from chia/flax) have shown anti-inflammatory properties that can support muscle recovery and body

composition improvement [47]. In conclusion, clinical evidence strongly supports the efficacy of the NNS formulation in improving body composition and cardiovascular health in endurance-trained individuals. Statistically significant improvements in muscle mass, BMI, weight, oxygen saturation, and heart rate demonstrated both metabolic and performance-related benefits. Although the fat percentage showed a slight increase, the overall improvements in lean mass and physiological metrics suggest a favorable shift in body composition. These findings, aligned with the current literature, validate the potential of well-designed, natural, multi-ingredient supplements for optimizing athletic health and performance. The NNS recipe seems able to reduce body composition and improve cardiopulmonary function, but with limits. Generalizability may be limited by the small sample size. Body composition can be measured using BIA, which is possible but susceptible to hydration-related measurement errors. Furthermore, the 8-week intervention period may be too short to reflect the long-term effects of the maintenance of these changes. Larger, long-term follow-up studies are required to validate these results.

5. Conclusions

The present findings strongly suggest that eight weeks of intervention with a new NNS has an impact on body composition and cardiopulmonary function in endurance-trained athletes. These findings emphasize the complementary properties of the supplements' main components (whey and pea proteins, fiber-rich flours, and nitrate-rich botanicals), which could support improved lean mass accretion, metabolic control, and cardiovascular function. A small increase in fat percentage was noted, although it is probably a relative artifact and a consequence of significant muscle mass gain. These results provide further evidence for the efficacy of a whole food source, a protein-rich supplement for use in sports nutrition, while presenting a safe and effective alternative for enhancing performance-related changes in body composition in active populations. These results support the potential of multi-ingredient natural supplements to improve athletic performance and suggest directions for future long-term studies. Further research with a larger sample size and a longer follow-up period is needed to investigate the long-term effects and mechanisms of treatment.

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