nutrients

MDPI

Article

Longitudinal Changes in Iron-Related Biomarkers, 25-Hydroxyvitamin D, Vitamin B12, Calcium, and Magnesium in Supplemented Male Runners and Triathletes Across a Competitive Season: A Prospective Single-Arm Study

Marcin Gierach [1,2,*] and Patrycja Victoria Czaj [3]

[1] Department of Endocrinology and Diabetology, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Toruń, ul. M. Skłodowskiej-Curie 9, 85-094 Bydgoszcz, Poland
[2] Cardiometabolic Center Gierach-Med. ul. Bydgoskich Olimpijczyków 5/39-40, 85-796 Bydgoszcz, Poland
[3] Endocrinology Student Research Club, Department of Endocrinology and Diabetology, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Toruń, ul. M. Skłodowskiej-Curie 9, 85-094 Bydgoszcz, Poland; 310531@stud.umk.pl
* Correspondence: marcin_gierach@wp.pl; Tel.: +48-507-180-677; Fax: +48-(052)-585-42-40

Abstract

Background/Objectives: Endurance athletes may be vulnerable to suboptimal micronutrient status because of dietary inadequacies, exercise-associated losses, and substantial training demands. This study primarily assessed longitudinal changes in iron-related biomarkers, 25-hydroxyvitamin D [25(OH)D], vitamin B12, calcium, and magnesium during a five-month physician-prescribed supplementation intervention in male runners and triathletes. Changes in body composition and metabolic parameters, as well as differences between athletic disciplines, were examined as secondary and exploratory outcomes. Methods: Fifty male endurance athletes (25 runners and 25 triathletes) underwent assessments in April 2024 (T0) and September 2024 (T1). All participants followed a physician-prescribed daily supplementation protocol comprising 40 mg elemental iron, 4000–6000 IU vitamin D3, 500 µg vitamin B12, 100 mg elemental magnesium, and 400–800 mg elemental calcium. The intervention was included in the approved research protocol. Linear mixed-effects models adjusted for age, baseline body mass index, and weekly training volume were used to evaluate longitudinal changes and time x group interactions. Benjamini–Hochberg false discovery rate correction (FDR) was applied within predefined outcome families. Results: Ferritin, serum iron, 25(OH)D, vitamin B12, calcium, and magnesium increased between T0 and T1 (all $q < 0.001$). Body mass, absolute fat mass, and fat mass percentage decreased, whereas fat-free mass and skeletal muscle mass remained stable. Visceral adipose tissue decreased at the nominal significance level but not after FDR correction ($p = 0.036$, $q = 0.080$). Fasting glucose, total cholesterol, LDL-C, non-HDL-C, triglycerides, alanine aminotransferase, and gamma-glutamyl transferase decreased, whereas HDL-C increased (all $q < 0.001$). Creatinine remained stable. No group effects or time × group interactions remained significant after FDR correction. Conclusions: Male runners and triathletes exhibited longitudinal improvements in several micronutrient-related biomarkers and concurrent changes in body composition and metabolic parameters during the five-month supplementation intervention. However, the absence of a non-supplemented control group prevents causal attribution of these changes to supplementation. The study does not establish optimal supplementation doses, improvements in athletic performance, or equivalence of responses between the two disciplines.

Check for updates

Academic Editor: Clemens Drenowatz

Received: 25 October 2026
Revised: 18 November 2026
Accepted: 19 November 2026
Published: 22 November 2026

Copyright: © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

Keywords: endurance exercise; metabolic biomarkers; body composition; micronutrient status; longitudinal monitoring; bioelectrical impedance analysis; supplementation intervention

1. Introduction

Endurance athletes are exposed to substantial and repeated physiological demands that increase the importance of adequate nutritional intake for maintaining health, supporting recovery, and sustaining training adaptations [1,2]. Although regular physical activity does not necessarily increase the recommended intake of every micronutrient, endurance athletes may be at increased risk of suboptimal micronutrient status because of high energy expenditure, insufficient dietary intake, restricted food choices, exercise-associated nutrient losses, and fluctuations in training load [1,3,4]. These risks are particularly relevant when nutritional intake does not adequately compensate for the demands of prolonged and repeated exercise. Accordingly, assessment of micronutrient status should distinguish between an increased risk of deficiency and a demonstrated need for higher supplementation doses. Correction of an identified deficiency may be beneficial, whereas routine supplementation in athletes with adequate baseline status does not necessarily confer additional physiological or performance advantages [3,5].

Iron is especially important in endurance exercise because it is required for erythropoiesis, hemoglobin-mediated oxygen transport, mitochondrial respiration, and oxidative energy production [5,6]. Reduced iron stores may occur before anemia develops and may be associated with fatigue, impaired exercise tolerance, and reduced capacity to adapt to training, particularly when iron depletion is pronounced [6,7]. Endurance athletes may be susceptible to disturbances in iron homeostasis because of gastrointestinal and urinary iron losses, sweating, mechanical hemolysis, increased erythrocyte turnover, and exercise-related inflammatory responses [5]. Furthermore, strenuous exercise may transiently increase circulating hepcidin, a key regulator of iron absorption and distribution, potentially reducing iron availability during the post-exercise period [5,8]. Supplementation studies indicate that oral iron can improve ferritin concentrations and other indicators of iron status, particularly in athletes with low baseline iron stores. However, improvements in biochemical status do not consistently translate into measurable performance benefits, and responses vary according to initial iron status, supplementation dose, treatment duration, and training characteristics [7,9].

Vitamin D is another micronutrient of interest in endurance athletes because of its role in calcium and phosphate homeostasis, bone metabolism, skeletal muscle function, and immune regulation. Circulating 25(OH)D concentrations may be influenced by dietary intake, supplementation, sunlight exposure, geographical location, and seasonal variation. Evidence from supplementation studies suggests that vitamin D administration can increase serum 25(OH)D concentrations, particularly in individuals with insufficient baseline status, although effects on exercise performance and muscle function remain inconsistent [10]. Importantly, high training loads alone do not establish a universal requirement for high-dose vitamin D supplementation. The appropriateness of supplementation should therefore be considered in relation to individual vitamin D status, clinical circumstances, dose, and safety.

Vitamin B12, magnesium, and calcium also participate in physiological processes relevant to endurance training. Vitamin B12 is essential for DNA synthesis, erythrocyte maturation, and normal neurological function. Although adequate vitamin B12 availability is necessary for hematological and metabolic health, evidence supporting additional supplementation in athletes with sufficient baseline status remains limited [11]. Magnesium contributes to ATP-dependent reactions, neuromuscular function, protein synthesis, and

muscle contraction. Some supplementation studies have suggested benefits for selected recovery-related outcomes, but findings remain heterogeneous and may depend on baseline magnesium status and the intervention protocol [12]. Calcium is fundamental to skeletal integrity, neuromuscular signaling, and muscle contraction, and adequate intake is particularly important in athletes exposed to substantial mechanical and metabolic demands. Nevertheless, circulating calcium is tightly regulated and may not accurately reflect dietary calcium adequacy or changes in skeletal calcium balance.

Despite the physiological relevance of these nutrients, interpretation of supplementation research in endurance athletes remains challenging. Published studies differ in participant characteristics, baseline micronutrient status, supplementation formulations and doses, intervention duration, training exposure, and measured outcomes [3,7,9,10,12]. Many investigations focus on individual micronutrients, while fewer examine concurrent changes in multiple biochemical markers during a standardized supplementation intervention. Moreover, an increase in circulating biomarker concentrations should not automatically be interpreted as evidence of improved physiological function, superior athletic performance, or an optimal supplementation strategy.

Comparisons between endurance disciplines may provide additional insight into longitudinal physiological responses. Long-distance runners and triathletes differ in the distribution of training across exercise modalities, mechanical loading, and typical weekly training volume. Such differences could influence body composition, iron metabolism, and other biochemical parameters during a competitive season. However, it remains uncertain whether these training characteristics are associated with different longitudinal biomarker patterns when athletes follow the same supplementation regimen. Seasonal changes in sunlight exposure, diet, and training must also be considered when interpreting measurements obtained at different points of a competitive season.

Therefore, the primary objective of the present study was to characterize longitudinal changes in ferritin, serum iron, 25(OH)D, vitamin B12, calcium, and magnesium concentrations during a five-month, physician-prescribed micronutrient supplementation intervention in male endurance athletes. The secondary objectives were to examine concurrent changes in body composition and selected metabolic and biochemical parameters and to explore whether the temporal patterns differed between long-distance runners and triathletes. Because the study used a prospective, single-arm design without a non-supplemented control group, the analyses were intended to describe changes occurring during the intervention rather than establish causal supplementation effects or determine optimal supplementation doses.

2. Materials and Methods

2.1. Bioethics

The study was conducted in accordance with the Declaration of Helsinki and approved by the Bioethics Committee of Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Toruń (approval No. KB 407/2023, dated 26 September 2023). The approved research protocol included the planned micronutrient supplementation intervention and specified the supplementation regimen and dosages administered during the study. Written informed consent was obtained from all participants before enrolment.

2.2. Study Design and Participants

This prospective, single-arm, uncontrolled supplementation intervention study was conducted during the 2024 competitive season. Fifty male endurance athletes were included in the longitudinal cohort, comprising 25 long-distance runners and 25 triathletes. All participants underwent two comprehensive assessments: the first in April 2024, at the

beginning of the competitive season (baseline, T0), and the second in September 2024, near the end of the competitive season (follow-up, T1). The observation period lasted five months.

The study protocol was originally submitted to the Bioethics Committee of Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Toruń, as an observational research project and received a favorable ethical opinion under this classification (approval No. KB 407/2023, dated 26 September 2023). The Committee did not require registration in a public clinical trial registry as part of the approval process; consequently, the study was not prospectively registered. In the present manuscript, the study is described as a prospective, single-arm, uncontrolled supplementation intervention to accurately reflect the investigator-directed nature of the supplementation regimen.

Participants were recruited between February and April 2024 through cooperating sports clubs and triathlon training groups. Information about the study and the eligibility criteria was distributed through these groups. Eligible participants were healthy male endurance athletes aged 26–58 years who had engaged in systematic endurance training for at least four years and regularly participated in running or triathlon competitions.

Exclusion criteria, assessed based on participant-reported medical history, included chronic diseases, regular medication use, and the use of prohibited performance-enhancing substances. All 50 athletes who volunteered for participation met the predefined eligibility criteria and were enrolled in the longitudinal cohort. Before enrolment, participants were asked about their previous use of dietary supplements. Athletes reported intermittent use of various supplements before the study, without following a uniform supplementation regimen. Previous supplement use was not an exclusion criterion.

Before inclusion, all participants were informed about the planned five-month supplementation protocol and agreed to take the prescribed supplements daily throughout the intervention period. Participants were responsible for administering the supplements themselves and agreed to remain in telephone contact with the research team for follow-up and adherence monitoring.

No formal a priori sample-size calculation was performed; the sample size was determined by the number of eligible athletes recruited during the predefined recruitment period. The study may have had limited statistical power to detect differences in longitudinal responses between the two athletic subgroups.

Participants reported typical weekly training volumes of approximately 6–8 h/week for runners and 10–14 h/week for triathletes (comprising approximately 3–4 h of swimming, 4–6 h of cycling, and 3–4 h of running). During the observation period, all participants continued their regular training programs under the supervision of their coaches. Both athletic subgroups followed the same physician-prescribed supplementation protocol, with individualized vitamin D3 and calcium doses determined on the basis of baseline biochemical findings. No non-supplemented control group was included.

The primary objective was to characterize longitudinal changes in iron-related and selected micronutrient biomarkers. Secondary objectives included changes in body composition and metabolic parameters, while comparisons of temporal responses between runners and triathletes were exploratory.

2.3. Supplementation Regimen

Following the baseline assessment (T0), all participants were prescribed a daily micronutrient supplementation regimen by a physician who was also a member of the research team. The intervention was implemented specifically for the purposes of the study, in accordance with the protocol approved by the Bioethics Committee. Vitamin D3 and calcium

doses were individually selected on the basis of baseline biochemical findings and the prescribing physician's clinical judgment.

The prescribed supplementation regimen consisted of:

- Feroplex® (Italfarmaco S.p.A., Milan, Italy) (15 mL/day; 40 mg elemental iron as ferric protein succinylate),
- magnesium citrate providing 100 mg elemental magnesium/day,
- vitamin D3 (4000–6000 IU/day),
- methylcobalamin (vitamin B12, 500 µg/day),
- Calperos® (Teva Pharmaceuticals Polska Sp. z o.o., Warsaw, Poland) 1000 (calcium carbonate; 1–2 capsules/day, corresponding to 400–800 mg elemental calcium/day)

Calcium supplementation was administered in the morning, whereas iron supplementation was scheduled after 15:00, with an interval of at least three hours between calcium and iron intake. The prescribed regimen was maintained throughout the approximately five-month intervention. Participants were instructed to follow this administration schedule throughout the intervention period.

Participants with baseline 25(OH)D concentrations $\leq$ 30 ng/mL (n = 30; 60%) were prescribed 6000 IU/day of vitamin D3, whereas those with concentrations $>$ 30 ng/mL (n = 20; 40%) were prescribed 4000 IU/day.

Calcium supplementation was adjusted according to baseline serum calcium concentrations. Participants with a baseline calcium concentration of 2.2 mmol/L (n = 17; 34%) were prescribed 800 mg/day of elemental calcium, whereas those with concentrations above 2.2 mmol/L (n = 33; 66%) were prescribed 400 mg/day.

The prescribed dose of 40 mg/day of elemental iron was selected based on the prescribing physician's clinical experience with endurance athletes undergoing intensive training and concerns regarding their increased susceptibility to iron depletion. This clinical rationale informed the decision to administer the same iron dose to all participants, including those without low baseline ferritin concentrations.

The prescribed vitamin D3 dosing range included doses exceeding the European Food Safety Authority's tolerable upper intake level of 4000 IU/day for adults; therefore, the regimen should not be interpreted as a general recommendation for endurance athletes.

Supplementation was self-administered by the participants. Adherence to the prescribed regimen was monitored through regular telephone follow-up conducted by the research team. During these contacts, participants were asked about their compliance with daily supplement intake and any difficulties associated with the regimen. As adherence was assessed by self-report rather than pill counts or standardized supplementation diaries, the proportion of prescribed doses actually consumed could not be objectively quantified.

During the intervention, clinical follow-up was provided by the study physician. Safety monitoring was based on medical interviews and participant-reported symptoms and supplement tolerability, including information obtained during telephone follow-up. No adverse symptoms were reported during these assessments. No interim biochemical safety measurements were performed; laboratory assessments were conducted only at the predefined baseline and follow-up visits.

The absence of interim biochemical monitoring limited the ability to detect potential asymptomatic abnormalities associated with vitamin D3, iron, and calcium supplementation. Furthermore, reliance on self-reported adherence and the lack of detailed records of previous supplement use limited the assessment of individual exposure and the attribution of observed biochemical changes to the prescribed intervention.

2.4. Anthropometric and Body Composition Assessment

Body height was measured to the nearest 0.1 cm using a calibrated wall-mounted stadiometer, and body mass was measured to the nearest 0.1 kg. Body mass index (BMI) was calculated as body mass divided by height squared (kg/m^2).

Body composition was assessed using a segmental multifrequency bioelectrical impedance analyzer (seca mBCA 515, seca GmbH & Co. KG, Hamburg, Germany) [13,14]. The device uses an eight-point bioelectrical impedance analysis system with multiple measurement frequencies to estimate body composition parameters. According to the manufacturer's recommendations, participants were measured barefoot while standing upright on the analyzer platform and holding the hand electrodes with their arms slightly abducted from the trunk. Measurements were performed under standardized laboratory conditions by the same trained investigator.

To minimize biological and environmental variability, all assessments were performed in the morning between 7:00 and 9:00 a.m. after an overnight fast. Participants were instructed to refrain from vigorous physical exercise for at least 24 h before testing and to avoid alcohol consumption for 48 h prior to the assessment. They were also asked to empty their urinary bladder immediately before the measurement. All measurements were obtained using the same device and following an identical standardized measurement protocol at both study visits.

The following anthropometric and body-composition variables were recorded and included in subsequent analyses: body mass, BMI, absolute fat mass (FM, kg), fat mass percentage (FM, %), fat-free mass (FFM, kg), skeletal muscle mass (MM, kg), total body water (TBW), extracellular water (ECW), the extracellular-to-total body water ratio (ECW/TBW), and visceral adipose tissue (VAT, L).

The standardized assessment conditions were intended to reduce measurement variability. Nevertheless, bioelectrical impedance estimates may be influenced by hydration status and fluid distribution, and this limitation was considered when interpreting longitudinal body-composition changes.

2.5. Blood Sampling and Biochemical Analyses

Venous blood samples were collected in the morning after an overnight fast and before any scheduled training session. To ensure standardized conditions, blood draws were performed between 9:00 and 10:00 a.m., immediately after the completion of the body composition assessments. Blood samples were collected at baseline (T0) and follow-up (T1). Follow-up blood samples were collected within seven days after completion of the five-month supplementation period. Follow-up appointments were scheduled according to participants' availability. Consequently, although blood sampling was performed under standardized morning fasting conditions, the interval between the last supplement intake and blood collection was not standardized across participants.

The following biochemical parameters were determined: glucose, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), creatinine, ferritin, serum iron, magnesium, calcium, vitamin B12, and 25(OH)D. Non-HDL cholesterol was calculated as total cholesterol minus HDL cholesterol.

Laboratory analyses were performed in a clinical laboratory using the cobas pro integrated solutions platform (Roche Diagnostics, Rotkreuz, Switzerland). Ferritin, vitamin B12, and 25(OH)D concentrations were measured using electrochemiluminescence immunoassays (ECLIA). Serum iron was determined using a ferrozine-based colorimetric method, while total calcium was measured using a photometric method. Magnesium concentrations were determined using a xylidyl blue colorimetric method. Glucose was measured using a

hexokinase-based enzymatic method, whereas total cholesterol was determined using an enzymatic colorimetric method.

HDL cholesterol and LDL cholesterol concentrations were measured using direct homogeneous enzymatic colorimetric methods, whereas triglycerides were determined using an enzymatic colorimetric method (GPO-PAP). Alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT) activities were measured using kinetic enzymatic methods in accordance with IFCC recommendations. Creatinine concentrations were determined using an enzymatic method.

Laboratory quality assurance included internal quality control (IQC) and participation in external quality assessment (EQA) programs. All measurements were performed in accordance with established laboratory analytical procedures and the manufacturers' instructions.

2.6. Statistical Analysis

Statistical analyses were performed using jamovi software, version 2.7.38 (The jamovi project, Sydney, Australia; based on R).

The distribution of continuous variables was assessed using the Shapiro–Wilk test, together with visual inspection of Q–Q plots and histograms. Continuous variables were summarized as means and standard deviations (SD), whereas categorical variables were presented as frequencies and percentages. Baseline comparisons between runners and triathletes were performed using independent-samples t-tests for approximately normally distributed variables and Mann–Whitney U tests for non-normally distributed variables.

Separate linear mixed-effects models (LMMs) were fitted for each body-composition and biochemical outcome using restricted maximum likelihood (REML) estimation. Participant identity was included as a random intercept to account for within-participant correlation between repeated measurements.

Time (T0 versus T1), athletic group (runners versus triathletes), and the time × group interaction were included as fixed effects. Age, baseline BMI, and weekly training volume (hours/week) were included as continuous covariates in adjusted models. Baseline BMI was omitted from the body-mass model because BMI is mathematically derived from body mass and height.

Categorical predictors were coded using centered contrasts (T0 = −0.5 and T1 = +0.5 for time; triathletes = −0.5 and runners = +0.5 for group), and continuous covariates were mean-centered. Accordingly, the main effect of time represented the longitudinal change averaged across athletic groups, whereas the main effect of group represented the adjusted between-group difference averaged across assessment points. The time × group interaction represented the difference in longitudinal changes between runners and triathletes.

Model assumptions were assessed by visual inspection of residual Q–Q plots and residual-versus-fitted plots. When potentially influential observations were identified, sensitivity analyses were performed by refitting the corresponding models after excluding both observations from the affected participant. All participants and observations were retained in the primary analyses. No observations were excluded from the primary models on the basis of residual diagnostics.

Multicollinearity among baseline predictors was assessed using variance inflation factors (VIFs) calculated from one baseline observation per participant. VIF values ranged from 1.11 to 2.21, indicating no evidence of substantial multicollinearity.

Additional sensitivity analyses were conducted for fat mass (kg) and total cholesterol by refitting the models without weekly training volume, without baseline BMI, and with age adjustment only. These analyses assessed the sensitivity of the estimated effects to alternative covariate specifications.

Fixed-effect estimates and corresponding 95% confidence intervals were reported. The Benjamini–Hochberg procedure was used to control the FDR within three outcome families: body-composition variables (nine outcomes), micronutrient-related biomarkers (six outcomes), and metabolic/biochemical parameters (nine outcomes).

Within each outcome family, FDR correction was applied separately to *p*-values for the main effect of time, the main effect of group, and the time × group interaction, resulting in nine separate correction sets encompassing 72 statistical tests. This approach was used to account for multiple comparisons within each outcome domain and model effect. Both nominal *p*-values and FDR-adjusted q-values were reported. Statistical significance after multiplicity correction was defined as $q < 0.05$.

No formal a priori sample-size calculation was performed. Given the uncontrolled study design, relatively small sample size, and multiple outcomes assessed, the analyses were interpreted as exploratory. In particular, non-significant time x group interactions were not considered evidence of equivalent responses between runners and triathletes.

3. Results

3.1. Participant Characteristics

Fifty male endurance athletes were included in the longitudinal analysis, comprising 25 runners and 25 triathletes. All participants completed both study visits, with no loss to follow-up during the approximately 5-month observation period. Baseline participant characteristics are presented in Table 1. The groups did not differ significantly in age, body mass, BMI, or training experience. Triathletes reported a significantly greater weekly training volume than runners. Multicollinearity diagnostics based on baseline data ($n = 50$) showed VIF values of 1.11 for age, 1.15 for baseline BMI, 2.21 for weekly training volume, and 2.12 for athletic group. These values did not indicate substantial multicollinearity among the predictors.

Table 1. Baseline demographic, anthropometric, and training characteristics of male endurance runners and triathletes.

Variable	Runners ($n = 25$)	Triathletes ($n = 25$)	*p*-Value
Age, years	39.32 ± 6.77	39.64 ± 8.34	0.882
Body mass, kg	79.43 ± 10.42	79.68 ± 9.35	0.803
BMI, kg/m^2	24.75 ± 2.51	23.70 ± 2.15	0.116
Training experience, years	10.72 ± 6.18	12.08 ± 4.54	0.072
Weekly training volume, h/week	8.20 ± 2.04	13.08 ± 2.69	<0.001

Values are presented as mean ± standard deviation (SD). Normality of data distribution was assessed using the Shapiro–Wilk test. Differences between the groups at baseline were evaluated using the independent-samples t-test for normally distributed variables (Age and BMI) and the Mann–Whitney U test for non-normally distributed variables (Body mass, training experience, and weekly training volume). Statistical significance was accepted at $p < 0.05$.

At baseline, 18 participants (36%) had serum ferritin concentrations below 30 ng/mL, compared with one participant (2%) at follow-up. No participant had ferritin concentrations below 15 ng/mL at either assessment, and none exceeded 200 ng/mL at follow-up. Furthermore, no participant exhibited serum iron or ferritin concentrations exceeding the laboratory reference ranges at follow-up.

Regarding vitamin D status, 2 participants (4%) had 25(OH)D concentrations below 20 ng/mL at baseline, while 25 participants (50%) had concentrations between 20 and <30 ng/mL. At follow-up, all participants had 25(OH)D concentrations ≥ 30 ng/mL. According to the Polish guidelines for vitamin D status assessment [15] 37 participants (74%)

had concentrations within the optimal range of 30–50 ng/mL, whereas 13 participants (26%) had concentrations > 50 ng/mL, classified as high values. The maximum observed 25(OH)D concentration was 62 ng/mL, and no participant exceeded 100 ng/mL.

Furthermore, no participant exhibited serum calcium concentrations exceeding the laboratory reference range at follow-up. The maximum observed serum calcium concentration was 2.5 mmol/L, recorded in two participants (4%).

These findings describe the distribution of biomarker concentrations and do not establish the clinical safety or efficacy of the supplementation regimen.

3.2. Changes in Body Composition

Descriptive statistics for body composition variables at baseline and follow-up during the 5-month observation period are summarized in Table 2. The results of the LMMs assessing the main effects of time, group, and their interaction (time × group) are presented in Table 3.

As shown in Table 3, significant changes in several body-composition parameters were observed over the 5-month observation period. A significant main effect of time was observed for body mass (estimate = −0.946 kg, 95% CI: −1.562 to −0.330; $p = 0.004$, q = 0.011), total fat mass (estimate = −1.170 kg, 95% CI: −1.823 to −0.517; $p < 0.001$, q = 0.004), and fat mass percentage (estimate = −1.218 percentage points, 95% CI: −1.899 to −0.537; $p < 0.001$, q = 0.004). Visceral adipose tissue showed a nominal decrease over time in the primary analysis (estimate = −0.123, 95% CI: −0.236 to −0.010; $p = 0.036$); however, this effect did not remain statistically significant after FDR correction (q = 0.080). No significant temporal changes were observed for fat-free mass, skeletal muscle mass, total body water, extracellular water, or the ECW/TBW ratio after FDR correction (all q > 0.05).

Sensitivity analyses were performed for outcomes showing potentially influential or extreme observations on residual diagnostics. Exclusion of the participant associated with an extreme residual in the fat-mass models did not materially alter the estimated temporal effects for fat mass (estimate = −1.106 kg, 95% CI: −1.760 to −0.451; $p = 0.002$) or fat mass percentage (estimate = −1.129 percentage points, 95% CI: −1.801 to −0.457; $p = 0.002$), supporting the robustness of the primary findings. In contrast, exclusion of the participant with an influential change in visceral adipose tissue attenuated the estimated time effect for VAT (estimate = −0.096, 95% CI: −0.197 to 0.006; $p = 0.067$). Thus, the nominal VAT change observed in the primary model should be interpreted cautiously.

Additional sensitivity analyses were performed to assess the influence of covariate adjustment on fat mass estimates. The main effect of time remained statistically significant across all model specifications, while the time × group interaction remained non-significant. In contrast, the estimated group effect varied depending on covariate adjustment: −1.559 kg in the fully adjusted model ($p = 0.306$), +0.951 kg after excluding weekly training volume ($p = 0.398$), −0.975 kg after excluding baseline BMI ($p = 0.642$), and +2.722 kg in the age-adjusted model ($p = 0.078$). These findings indicate that the estimated between-group difference in fat mass was sensitive to covariate selection, whereas the longitudinal time effect was more robust.

No statistically significant time × group interactions were observed for any of the analyzed body-composition or fluid-distribution variables after FDR correction (all q > 0.05), indicating no statistical evidence of differential temporal changes between runners and triathletes. Likewise, no significant main effects of group remained after FDR correction (all q > 0.05).

Table 2. Descriptive statistics of body composition and fluid distribution parameters in endurance athletes at baseline and follow-up.

Time	Group	BM (kg)	FM (kg)	TBW (L)	ECW/TBW %	ECW (L)	VAT (L)	MM (kg)	FFM (kg)	FM (%)
T0 (baseline)	T	79.7 ± 9.35	12.6 ± 5.23	49.2 ± 4.97	40.3 ± 0.951	19.9 ± 2.12	1.15 ± 0.692	33.1 ± 3.69	67.1 ± 6.46	15.5 ± 4.87
	R	79.4 ± 10.4	15.3 ± 6.18	46.8 ± 4.28	40.7 ± 1.27	19.1 ± 1.93	1.51 ± 0.873	31.2 ± 3.17	64.1 ± 5.69	18.8 ± 5.04
T1 (follow-up)	T	78.7 ± 8.99	11.5 ± 4.63	49.3 ± 5.40	40.5 ± 1.10	20.0 ± 2.44	0.998 ± 0.524	33.0 ± 3.89	67.2 ± 7.12	14.4 ± 4.84
	R	78.5 ± 10.1	14.1 ± 5.76	47.1 ± 4.11	40.7 ± 1.26	19.2 ± 1.93	1.42 ± 0.813	31.4 ± 3.03	64.4 ± 5.52	17.5 ± 4.76

Values are presented as mean ± standard deviation (SD). Abbreviations: T—Triathletes; R—Runners; BM—Body Mass; FM—Fat Mass; VAT—Visceral Adipose Tissue; MM—Muscle Mass; FFM—Fat-Free Mass; TBW—Total Body Water; ECW—Extracellular Water; ECW/TBW %—Extracellular Water to Total Body Water %.

Table 3. Results of the linear mixed-effects models (LMM) for body composition and fluid distribution variables.

Outcome	Effect	Estimate (95% CI)	*p*-Value	q-Value
BM (kg)	Time	−0.946 (−1.562 to −0.330)	0.004	0.011
	Group	−4.592 (−12.395 to 3.212)	0.249	0.394
	Time × Group	0.116 (−1.116 to 1.348)	0.852	0.858
FM (kg)	Time	−1.170 (−1.823 to −0.517)	<0.001	0.004
	Group	−1.559 (−4.551 to 1.433)	0.306	0.394
	Time × Group	−0.134 (−1.440 to 1.172)	0.839	0.858
FM (%)	Time	−1.218 (−1.899 to −0.537)	<0.001	0.004
	Group	−0.374 (−3.435 to 2.686)	0.809	0.885
	Time × Group	−0.220 (−1.583 to 1.143)	0.750	0.858
VAT (L)	Time	−0.123 (−0.236 to −0.010)	0.036	0.080
	Group	−0.228 (−0.646 to 0.191)	0.285	0.394
	Time × Group	0.062 (−0.164 to 0.288)	0.588	0.858
FFM (kg)	Time	0.227 (−0.145 to 0.598)	0.232	0.272
	Group	−4.214 (−8.518 to 0.091)	0.058	0.142
	Time × Group	0.248 (−0.495 to 0.992)	0.510	0.858
MM (kg)	Time	0.067 (−0.141 to 0.275)	0.524	0.524
	Group	−2.550 (−4.923 to −0.176)	0.038	0.142
	Time × Group	0.301 (−0.114 to 0.716)	0.156	0.844
TBW (L)	Time	0.178 (−0.105 to 0.461)	0.218	0.272
	Group	−3.354 (−6.631 to −0.076)	0.048	0.142
	Time × Group	0.204 (−0.363 to 0.771)	0.478	0.858
ECW (L)	Time	0.118 (−0.036 to 0.272)	0.135	0.244
	Group	−1.404 (−2.869 to 0.061)	0.063	0.142
	Time × Group	−0.028 (−0.337 to 0.281)	0.858	0.858
ECW/TBW (%)	Time	0.094 (−0.064 to 0.252)	0.242	0.272
	Group	−0.059 (−0.858 to 0.740)	0.885	0.885
	Time × Group	−0.212 (−0.527 to 0.103)	0.188	0.844

Estimates represent adjusted fixed-effect coefficients with corresponding 95% confidence intervals (CI). Time was coded as T1 (follow-up) versus T0 (baseline), and group as runners versus triathletes. All models included participant identity as a random intercept and were adjusted for age, baseline BMI, and weekly training volume, except for the body mass model, in which baseline BMI was omitted. Nominal *p*-values and Benjamini–Hochberg FDR-adjusted q-values are presented. FDR correction was applied separately to the *p*-values for the main effect of time, group, and the time × group interaction across the nine body-composition outcomes. Statistical significance after correction was defined as $q < 0.05$. Abbreviations: BM—body mass; FM—fat mass; VAT—visceral adipose tissue; FFM—fat-free mass; MM—skeletal muscle mass; TBW—total body water; ECW—extracellular water; ECW/TBW—extracellular-to-total body water ratio; CI—confidence interval; FDR—false discovery rate.

3.3. Changes in Iron-Related and Micronutrient Biomarkers

The descriptive statistics for iron-related and micronutrient biomarkers are presented in Table 4, whereas the adjusted LMMs results are shown in Table 5.

Table 4. Descriptive statistics of iron-related and micronutrient biomarkers at baseline (T0) and follow-up (T1) during the 5-month observation period.

Time	Group	Ferritin (ng/mL)	Serum Iron (µg/dL)	25(OH)D (ng/mL)	Vitamin B12 (pg/mL)	Ca (mmol/L)	Mg (mmol/L)
T0 (baseline)	T	36.48 ± 8.84	93.30 ± 12.10	30.70 ± 4.53	331 ± 58.6	2.28 ± 0.06	0.856 ± 0.038
	R	30.80 ± 8.35	90.60 ± 8.72	27.60 ± 5.77	314 ± 61.3	2.27 ± 0.07	0.867 ± 0.046
T1 (follow-up)	T	53.10 ± 9.65	105.00 ± 9.37	46.20 ± 6.05	534 ± 78.5	2.40 ± 0.03	0.944 ± 0.034
	R	48.30 ± 10.70	105.00 ± 11.50	43.80 ± 7.88	507 ± 76.7	2.39 ± 0.04	0.950 ± 0.033

Values are presented as mean ± standard deviation (SD). Each subgroup comprised 25 participants. Abbreviations: T—triathletes; R—runners; 25(OH)D—25-hydroxyvitamin D; Ca—calcium; Mg—magnesium.

In adjusted LMMs, ferritin, serum iron, 25(OH)D, vitamin B12, calcium, and magnesium all increased significantly over time (all $p < 0.001$; all q < 0.001 after FDR correction).

No statistically significant main effect of group was observed after FDR correction for ferritin (q = 0.990), serum iron (q = 0.990), 25(OH)D (q = 0.990), vitamin B12 (q = 0.990), calcium (q = 0.930), or magnesium (q = 0.138). Likewise, no statistically significant time × group interactions were observed for any assessed micronutrient-related biomarker after FDR correction (all q = 0.833), indicating that there was no statistical evidence that the temporal changes differed between runners and triathletes.

Figure 1 illustrates the estimated marginal means with 95% confidence intervals for selected micronutrient-related biomarkers.

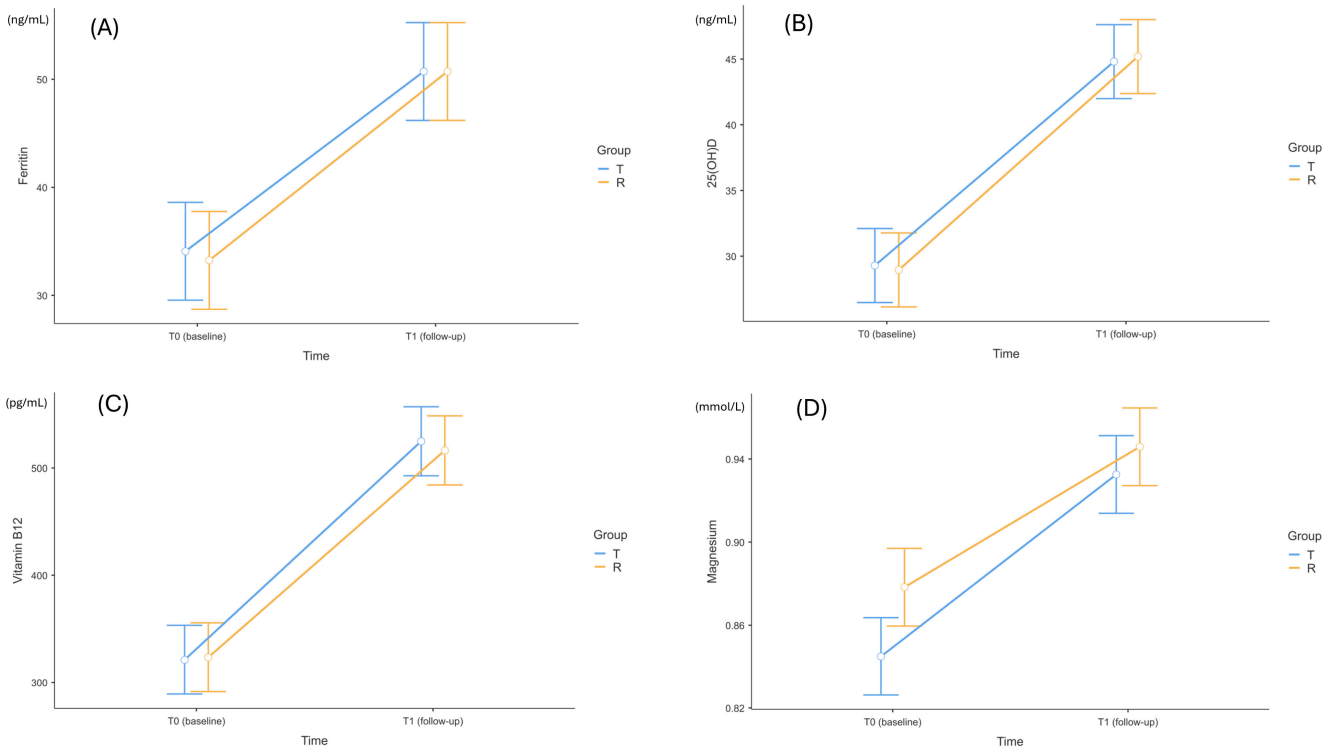

Figure 1. Estimated marginal means with 95% confidence intervals illustrating longitudinal changes in selected micronutrient-related biomarkers during the 5-month observation period. (**A**) Ferritin (ng/mL); (**B**) 25-hydroxyvitamin D [25(OH)D] (ng/mL); (**C**) Vitamin B12 (pg/mL); (**D**) Magnesium (mmol/L). Blue lines and markers represent triathletes (T), whereas orange lines and markers represent runners (R). T0 denotes baseline and T1 denotes follow-up. All displayed biomarkers increased significantly over time (all $p < 0.001$; all q < 0.001), whereas no statistically significant time × group interactions were observed.

Table 5. Results of the linear mixed-effects models (LMM) for iron-related and micronutrient biomarkers.

Variable	Time Estimate (95% CI)	*p*	q	Group Estimate (95% CI)	*p*	q	Time × Group Estimate (95% CI)	*p*	q
Ferritin (ng/mL)	17.06 (15.24, 18.88)	<0.001	<0.001	−0.42 (−7.50, 6.66)	0.907	0.990	0.84 (−2.81, 4.49)	0.649	0.833
Serum iron (µg/dL)	13.22 (10.47, 15.97)	<0.001	<0.001	2.27 (−5.24, 9.78)	0.551	0.990	3.16 (−2.35, 8.67)	0.260	0.833
25(OH)D (ng/mL)	15.88 (14.11, 17.65)	<0.001	<0.001	0.03 (−4.11, 4.16)	0.990	0.990	0.72 (−2.82, 4.26)	0.688	0.833
Vitamin B12 (pg/mL)	198.32 (179.16, 217.48)	<0.001	<0.001	−3.13 (−50.93, 44.68)	0.897	0.990	−10.80 (−49.12, 27.52)	0.578	0.833
Calcium (mmol/L)	0.118 (0.099, 0.137)	<0.001	<0.001	0.016 (−0.015, 0.046)	0.310	0.930	0.004 (−0.033, 0.041)	0.833	0.833

Table 5. *Cont.*

Variable	Time Estimate (95% CI)	*p*	q	Group Estimate (95% CI)	*p*	q	Time × Group Estimate (95% CI)	*p*	q
Magnesium (mmol/L)	0.086 (0.075, 0.096)	<0.001	<0.001	0.032 (0.005, 0.058)	0.023	0.138	−0.004 (−0.024, 0.016)	0.699	0.833

Values represent fixed-effect estimates with corresponding 95% confidence intervals (95% CIs) from separate linear mixed-effects models. Time estimates represent the adjusted mean change from baseline to follow-up (T1 − T0), group estimates represent the adjusted difference between runners and triathletes (R − T), and time × group estimates represent the between-group difference in temporal change. Each model included time (baseline (T0) vs. follow-up (T1)), group (runners vs. triathletes), and the time × group interaction as fixed effects; participant identity was included as a random intercept. Models were adjusted for age, baseline body mass index (BMI), and weekly training volume (hours/week). Models were estimated using restricted maximum likelihood (REML), with degrees of freedom calculated using the Satterthwaite approximation. q-values were obtained using the Benjamini–Hochberg false discovery rate (FDR) procedure within the micronutrient biomarker family, applied separately to the *p*-values for the main effect of time, the main effect of group, and the time × group interaction. For analyses subjected to FDR correction, statistical significance was defined as $q < 0.05$.

3.4. Changes in Biochemical Markers

Changes in biochemical and metabolic variables over the 5-month observation period are summarized in Table 6, while the corresponding LMM estimates, adjusted for baseline BMI, age, and weekly training volume, are presented in Table 7.

The LMM analysis revealed a significant main effect of time for fasting glucose (estimate = −1.740 mg/dL, 95% CI: −2.548 to −0.932; $p < 0.001$, $q < 0.001$), indicating a modest decrease over the 5-month observation period. A nominal main effect of group was observed ($p = 0.049$), but it did not remain statistically significant after FDR correction ($q = 0.131$). The time × group interaction was not statistically significant ($p = 0.061$, $q = 0.549$), providing no evidence of differential temporal changes between runners and triathletes.

Significant main effects of time were observed for all lipid parameters. Total cholesterol (estimate = −9.440 mg/dL, 95% CI: −12.787 to −6.093), LDL-C (estimate = −9.040 mg/dL, 95% CI: −11.628 to −6.452), non-HDL-C (estimate = −12.880 mg/dL, 95% CI: −16.773 to −8.987), and triglycerides (estimate = −16.440 mg/dL, 95% CI: −20.701 to −12.180) decreased over time, whereas HDL-C increased (estimate = 3.440 mg/dL, 95% CI: 2.429 to 4.451); all time effects remained statistically significant after FDR correction (all $p < 0.001$, $q < 0.001$). Nominal group effects were observed for non-HDL-C ($p = 0.043$) and triglycerides ($p = 0.032$), but neither remained statistically significant after FDR correction (both $q = 0.131$). No significant time × group interactions were observed for any lipid parameter after FDR correction (all $q > 0.05$). The longitudinal trajectories of fat mass and selected lipid parameters are illustrated in Figure 2, showing the corresponding estimated marginal means and 95% confidence intervals for runners and triathletes.

Additional sensitivity analyses were conducted to evaluate the influence of covariate adjustment on total cholesterol estimates. The main effect of time remained statistically significant across all model specifications, and the time × group interaction remained non-significant. However, the estimated group effect varied substantially depending on covariate adjustment: −12.139 mg/dL in the fully adjusted model ($p = 0.058$), +0.157 mg/dL after excluding weekly training volume ($p = 0.974$), −10.526 mg/dL after excluding baseline BMI ($p = 0.160$), and +5.242 mg/dL in the age-adjusted model ($p = 0.343$). These results suggest that the estimated between-group difference in total cholesterol was sensitive to covariate selection, whereas the longitudinal time effect was comparatively robust.

Table 6. Descriptive statistics of biochemical and metabolic parameters in endurance athletes at baseline and follow-up.

Time	Group	Glucose (mg/dL)	Creatinine (mg/dL)	TC (mg/dL)	LDL-C (mg/dL)	HDL-C (mg/dL)	TG (mg/dL)	ALT (U/L)	N-HDL (mg/dL)	GGT (U/L)
T0 (baseline)	T	86.7 ± 5.05	0.869 ± 0.042	196 ± 23.1	119 ± 21.0	48.7 ± 6.49	113 ± 30.1	22.7 ± 7.62	148 ± 24.3	33.2 ± 8.06
	R	88.9 ± 5.76	0.883 ± 0.053	202 ± 23.6	129 ± 22.8	47.2 ± 5.01	122 ± 29.9	26.2 ± 9.29	155 ± 26.4	32.9 ± 6.99
T1 (follow-up)	T	85.8 ± 3.97	0.866 ± 0.039	188 ± 15.0	112 ± 17.6	51.4 ± 6.39	98.0 ± 23.7	19.4 ± 6.06	136 ± 17.5	30.9 ± 7.09
	R	86.4 ± 4.69	0.881 ± 0.050	192 ± 17.4	119 ± 17.6	51.3 ± 5.17	104 ± 19.8	22.1 ± 7.34	141 ± 18.9	30.7 ± 6.98

Values are presented as mean ± standard deviation (SD). T0 = baseline; T1 = follow-up; T—Triathletes; R—Runners; TC—Total cholesterol; LDL-C—Low-density lipoprotein cholesterol; HDL-C—High-density lipoprotein cholesterol; TG—Triglycerides; ALT—Alanine aminotransferase; N-HDL—Non-high-density lipoprotein cholesterol; GGT—Gamma-glutamyl transferase.

Table 7. Fixed-effect estimates from linear mixed-effects models (LMM) for biochemical and metabolic markers.

Variable	Time Estimate (95% CI)	*p*	q	Group Estimate (95% CI)	*p*	q	Time × Group Estimate (95% CI)	*p*	q
Glucose (mg/dL)	−1.740 (−2.548 to −0.932)	<0.001	<0.001	−3.129 (−6.196 to −0.061)	0.049	0.131	−1.560 (−3.176 to 0.056)	0.061	0.549
Total cholesterol (mg/dL)	−9.440 (−12.787 to −6.093)	<0.001	<0.001	−12.139 (−24.543 to 0.265)	0.058	0.131	−1.600 (−8.294 to 5.094)	0.637	0.819
LDL-C (mg/dL)	−9.040 (−11.628 to −6.452)	<0.001	<0.001	−9.672 (−22.638 to 3.294)	0.145	0.218	−2.400 (−7.576 to 2.776)	0.362	0.681
HDL-C (mg/dL)	3.440 (2.429 to 4.451)	<0.001	<0.001	2.068 (−2.423 to 6.558)	0.365	0.411	1.360 (−0.661 to 3.381)	0.188	0.681
Non-HDL-C (mg/dL)	−12.880 (−16.773 to −8.987)	<0.001	<0.001	−14.206 (−27.737 to −0.675)	0.043	0.131	−2.960 (−10.746 to 4.826)	0.454	0.681
Triglycerides (mg/dL)	−16.440 (−20.701 to −12.180)	<0.001	<0.001	−18.733 (−35.525 to −1.940)	0.032	0.131	−3.840 (−12.363 to 4.680)	0.375	0.681
ALT (U/L)	−3.660 (−4.635 to −2.685)	<0.001	<0.001	−4.265 (−9.094 to 0.565)	0.086	0.155	−0.760 (−2.710 to 1.190)	0.443	0.681
GGT (U/L)	−2.240 (−3.084 to −1.396)	<0.001	<0.001	−3.609 (−8.910 to 1.691)	0.183	0.235	0.000 (−1.688 to 1.688)	1.000	1.000
Creatinine (mg/dL)	−0.002 (−0.010 to 0.006)	0.588	0.588	−0.010 (−0.042 to 0.023)	0.565	0.565	0.001 (−0.015 to 0.017)	0.882	0.992

Values represent adjusted fixed-effect estimates with corresponding 95% confidence intervals (CI). Time was coded as T1 (follow-up) versus T0 (baseline), and group as runners versus triathletes. All models included participant identity as a random intercept and were adjusted for age, baseline body mass index (BMI), and weekly training volume (hours/week). Nominal *p*-values and Benjamini–Hochberg FDR-adjusted q-values are presented. FDR correction was applied separately to the *p*-values for the main effect of time, the main effect of group, and the time × group interaction across the nine metabolic/biochemical outcomes. Statistical significance after correction was defined as $q < 0.05$.

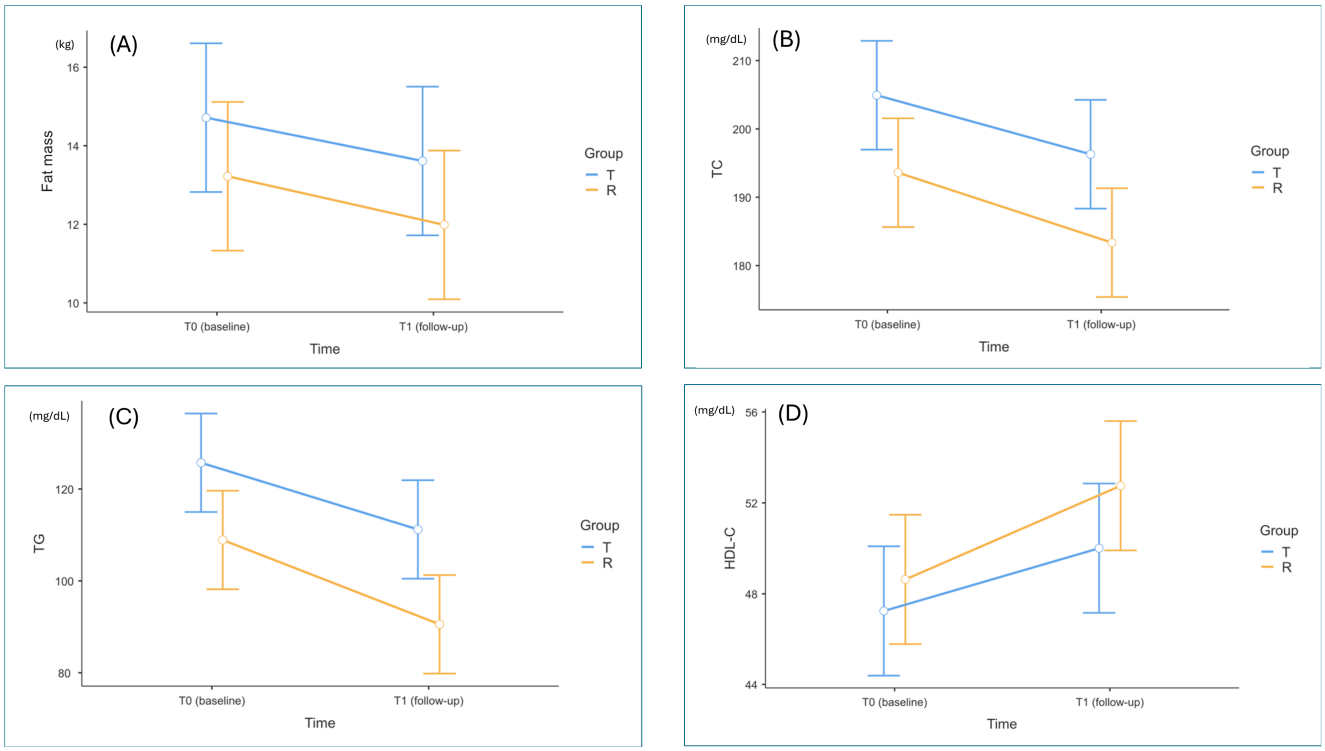

Figure 2. Estimated marginal means with 95% confidence intervals showing longitudinal changes over the 5-month observation period. (**A**) Fat Mass (kg); (**B**) Total Cholesterol (mg/dL); (**C**) Triglycerides (mg/dL); (**D**) High-Density Lipoprotein Cholesterol (HDL-C, mg/dL). Orange lines/markers represent runners (R), and blue lines/markers represent triathletes (T). All displayed outcomes showed significant main effects of time after FDR correction ($q < 0.05$), with no significant time × group interactions. The estimated marginal means represent model-based predictions adjusted for age, baseline BMI, and weekly training volume, with continuous covariates held at their sample means. Accordingly, these adjusted estimates may differ from the unadjusted descriptive means presented in Tables 2 and 6, including in the direction of between-group differences. Abbreviations: TC—total cholesterol; TG—triglycerides; HDL-C—high-density lipoprotein cholesterol; BMI—body mass index; FDR—false discovery rate; R—runners; T—triathletes; T0—baseline; T1—follow-up.

Significant main effects of time were also observed for ALT (estimate = −3.660 U/L, 95% CI: −4.635 to −2.685; $p < 0.001$, $q < 0.001$) and GGT (estimate = −2.240 U/L, 95% CI: −3.084 to −1.396; $p < 0.001$, $q < 0.001$), both of which decreased over the observation period. No significant group or time × group effects remained after FDR correction for either hepatic marker (all $q > 0.05$). Serum creatinine remained stable over time (estimate = −0.002 mg/dL, 95% CI: −0.010 to 0.006; $p = 0.588$, $q = 0.588$), with no significant group effect ($p = 0.565$, $q = 0.565$) or time × group interaction ($p = 0.882$, $q = 0.992$).

4. Discussion

4.1. Principal Findings

The present prospective, single-arm, uncontrolled supplementation intervention investigated longitudinal changes in micronutrient-related biomarkers, body composition, and metabolic parameters in male runners and triathletes over a five-month competitive season. Four principal findings emerged. First, significant increases were observed in ferritin and serum iron concentrations, alongside higher circulating concentrations of 25(OH)D, vitamin B12, magnesium, and calcium. Second, significant reductions in body mass, fat mass, and body fat percentage were observed, whereas fat-free mass, skeletal muscle mass, and body water parameters did not exhibit statistically significant changes after correction for multiple

testing. Third, significant temporal changes were observed in several metabolic indicators, including decreases in fasting glucose, total cholesterol, LDL-C, non-HDL-C, triglycerides, ALT, and GGT, alongside an increase in HDL-C, while serum creatinine remained stable. Finally, no statistically significant differences in longitudinal responses between runners and triathletes were detected after FDR correction, although this finding does not establish equivalent responses between the two disciplines.

These findings provide an integrated overview of biochemical and body-composition changes occurring during a standardized, investigator-prescribed supplementation intervention. Previous investigations have examined individual micronutrients, including iron, vitamin D, and magnesium, in endurance athletes [16–18]. Building on these investigations, the present study assessed concurrent longitudinal changes across multiple physiological domains, including micronutrient status, body composition, and metabolic biomarkers. Although both athletic groups received the same prescribed supplementation regimen, they differed substantially in weekly training volume and training characteristics. Sensitivity analyses indicated that the estimated between-group differences in fat mass and total cholesterol were influenced by covariate selection, whereas the corresponding longitudinal time effects were more robust. Therefore, the absence of statistically significant time × group interactions should be interpreted cautiously, particularly given the relatively small sample size and exploratory nature of these comparisons.

Importantly, the observed changes cannot be attributed specifically to supplementation because the study did not include a non-supplemented control group. Variations in habitual dietary intake, training exposure, sunlight availability, and other seasonal or physiological factors may have contributed to the longitudinal trajectories. Furthermore, increases in circulating micronutrient concentrations do not necessarily indicate improved nutritional adequacy, physiological function, or athletic performance. Accordingly, the findings should be interpreted as descriptive longitudinal changes occurring during the intervention rather than evidence of supplementation efficacy or optimal dosing. The following sections discuss these observations in relation to previous research, potential physiological mechanisms, and the methodological limitations of the study.

4.2. Iron Metabolism in Endurance Athletes

The present study demonstrated significant increases in ferritin and serum iron concentrations during the five-month competitive season, with adjusted mean changes of 17.06 ng/mL and 13.22 µg/dL, respectively (both $q < 0.001$). The proportion of athletes with ferritin concentrations below 30 ng/mL decreased from 36% (18/50) at baseline to 2% (1/50) at follow-up. Neither the main group effect nor the time × group interaction was statistically significant after FDR correction. These findings are clinically relevant given the susceptibility of endurance athletes to disturbances in iron homeostasis associated with exercise-induced hemolysis, gastrointestinal losses, increased erythropoietic demand, and inflammatory responses. McCormick et al. reported a comparable ferritin increase of 19.7 µg/L over eight weeks in 31 endurance runners with suboptimal baseline iron status. However, their participants received 105 mg of elemental iron daily or on alternate days, compared with 40 mg daily in our cohort. Differences in baseline iron status, supplementation dose, and study design preclude conclusions regarding comparable efficacy, particularly in the absence of a non-supplemented control group in our study [16].

Further evidence comes from Córdova et al. [19], who evaluated iron supplementation in 18 elite male cyclists during an intensive competitive period. Unlike our single-arm study, their investigation included a non-supplemented comparison group. Ferritin increased from 153.3 ± 68.8 to 231.0 ± 62.0 ng/mL in supplemented cyclists but decreased from 128.3 ± 55.7 to 104.7 ± 41.2 ng/mL in non-supplemented participants, with a significant time × group

interaction ($p = 0.004$). Serum iron also showed a significant interaction ($p = 0.018$), remaining relatively stable in supplemented athletes while declining in controls. This comparison provides stronger evidence of differing iron-related biomarker trajectories between supplemented and non-supplemented athletes. However, substantially higher baseline ferritin concentrations, a greater elemental iron dose (80 mg/day), and the demanding three-week stage-race setting limit direct comparisons with our five-month intervention.

In contrast, Ishibashi et al. [20] reported no statistically significant longitudinal changes in ferritin or serum iron concentrations among 16 highly trained female long-distance runners assessed during regular and intensified training periods. Ferritin changed from 30.9 ± 22.2 to 28.1 ± 11.8 ng/mL, while serum iron changed from 55 ± 24 to 65 ± 8 µg/dL, with neither change reaching statistical significance. However, hepcidin concentrations increased significantly from 8.8 ± 6.1 to 16.3 ± 6.5 ng/mL ($p = 0.027$). As hepcidin regulates intestinal iron absorption and iron recycling, these findings suggest that intensified training may alter iron regulation without corresponding significant changes in conventional iron biomarkers. Differences in sex, training conditions, and supplementation practices limit direct comparisons with our male cohort. Moreover, neither hepcidin nor inflammatory markers were measured in our study, preventing assessment of their potential contribution to the observed changes in ferritin and serum iron.

The influence of training exposure and blood-sampling timing is further illustrated by studies examining shorter-term or intensified exercise periods. Manechini et al. [21] found no significant changes in serum iron across a 12-week marathon training program but observed a marked increase immediately after marathon completion. Similarly, Bizjak et al. [22] reported a significant decrease in ferritin ($p < 0.0001$) following a one-week endurance training camp characterized by substantially increased training volume. Together, these studies suggest that iron-related biomarkers may exhibit different trajectories depending on training intensity, recovery conditions, and sampling schedules, which may partly explain differences between their findings and those observed in our cohort. Importantly, acute post-exercise fluctuations and responses to short periods of intensified training should not be directly equated with longitudinal changes observed over a five-month competitive season.

The simultaneous increases in ferritin and serum iron observed in our cohort are consistent with favorable changes in circulating iron-related biomarkers, but their physiological interpretation remains limited. Ferritin is also an acute-phase reactant, and an increase in its concentration does not necessarily reflect a proportional increase in body iron stores. The absence of complementary iron indices, such as transferrin saturation, further limits the assessment of iron status. Moreover, dietary intake, training load, recovery, and seasonal factors may have contributed to the observed changes independently of the prescribed supplementation regimen. From a clinical perspective, these findings support interpreting ferritin alongside complementary laboratory markers and the athlete's individual clinical and training context rather than in isolation.

4.3. Vitamin D and Serum Calcium Status in Endurance Athletes

Serum 25(OH)D concentrations increased significantly over the five-month competitive season, with an adjusted mean increase of 15.88 ng/mL (95% CI: 14.11–17.65; $q < 0.001$). At baseline, 27 of 50 athletes (54%) had 25(OH)D concentrations below 30 ng/mL, whereas all participants reached concentrations of at least 30 ng/mL at follow-up. Neither the main group effect nor the time × group interaction was statistically significant after FDR correction. Although these findings indicate a substantial improvement in circulating vitamin D status during follow-up, they do not establish equivalent responses between disciplines or demonstrate that supplementation alone accounted for the observed increase.

The observed increase in 25(OH)D should be interpreted in the context of the April-to-September observation period, during which greater exposure to ultraviolet B radiation may have enhanced endogenous vitamin D synthesis, particularly among athletes training outdoors. The importance of seasonal variation was demonstrated by Krzywanski et al. [23], who assessed 409 Polish elite athletes and reported higher mean vitamin D concentrations in outdoor athletes during summer than winter (36 ± 1 vs. 25 ± 1 ng/mL). Furthermore, winter training camps involving increased sunlight exposure were associated with a rise in 25(OH)D from approximately 25 to 46 ng/mL. These findings indicate that seasonal and environmental factors may substantially influence vitamin D concentrations independently of supplementation.

Conversely, Wilson-Barnes et al. [24] observed a significant decline in 25(OH)D concentrations among 47 competitive university athletes from autumn to spring (56.1 ± 23.3 to 31.0 ± 16.5 nmol/L; $p < 0.001$), affecting both indoor and outdoor athletes. Together with the findings of Krzywanski et al., these contrasting seasonal trajectories demonstrate that vitamin D status may vary substantially across the year, irrespective of regular athletic training. Consequently, the increase observed in our cohort during spring and summer may partly reflect changes in sunlight exposure. Because individual ultraviolet exposure was not assessed, its contribution cannot be separated from that of the investigator-prescribed supplementation regimen.

Supplementation represents another relevant factor when interpreting the present findings. Gervasi et al. [17] investigated the effects of 2000 IU/day of vitamin D3 over eight weeks in recreational runners and non-runners during autumn and winter. Among supplemented runners, serum 25(OH)D increased from 30.45 ± 7.0 to 35.35 ± 8.9 ng/mL ($p < 0.001$), whereas no significant change was observed in non-supplemented runners. Similarly, Kasprowicz et al. [25] reported that vitamin D supplementation maintained 25(OH)D concentrations in ultramarathon runners, whereas concentrations decreased significantly in the placebo group. Unlike these controlled investigations, our study involved individually prescribed vitamin D3 doses of 4000–6000 IU/day, selected according to baseline 25(OH)D concentrations and clinical judgment, over five months without a non-supplemented comparator. Differences in supplementation dose, intervention duration, seasonal conditions, and study design limit direct comparisons and prevent the observed increase in our cohort from being attributed specifically to supplementation.

The importance of training and environmental context is further illustrated by Bizjak et al. [22], who reported a significant increase in circulating 25(OH)D concentrations ($p < 0.0001$) following a one-week endurance training camp conducted in a sunny environment. Although the shorter observation period and intensified training conditions limit direct comparability with our five-month study, these findings further highlight the importance of accounting for environmental exposure and training-related factors when interpreting longitudinal vitamin D changes.

Beyond its established role in calcium homeostasis and skeletal health, vitamin D has also been investigated in relation to muscle function and athletic performance. However, Gervasi et al. [17] reported no significant improvements in maximal oxygen uptake or countermovement jump performance despite increased circulating 25(OH)D concentrations. Similarly, Wilson-Barnes et al. [24] did not demonstrate consistent improvements in physical performance associated with seasonal vitamin D status. These findings indicate that biochemical changes in 25(OH)D should not automatically be interpreted as functional or ergogenic benefits. As physical performance, bone turnover, and muscle function were not assessed as longitudinal outcomes in our study, such benefits cannot be inferred from the observed biomarker changes.

Serum calcium concentrations also increased significantly during follow-up, with an adjusted mean increase of 0.118 mmol/L (95% CI: 0.099–0.137; q < 0.001). However, circulating calcium is tightly regulated and does not directly reflect dietary calcium intake, calcium absorption, or total-body calcium stores. Gervasi et al. [17] reported a significant overall time effect for serum calcium but no significant time × supplementation interaction ($p = 0.593$), despite changes in vitamin D concentrations. These findings suggest that circulating calcium and 25(OH)D do not necessarily follow parallel trajectories. Accordingly, their concurrent increases in our cohort cannot establish a direct physiological or causal relationship.

An important consideration is the investigator-prescribed supplementation regimen, which included individually selected doses of 4000–6000 IU/day of vitamin D3 and 400–800 mg/day of elemental calcium over five months. This range included doses exceeding the tolerable upper intake level of 4000 IU/day established by the European Food Safety Authority (EFSA) [26]. At follow-up, 13 participants (26%) had 25(OH)D concentrations of at least 50 ng/mL, including one participant with a concentration of 62 ng/mL. Although these findings do not establish vitamin D toxicity, they warrant cautious interpretation of the dosing regimen. The absence of detailed dietary intake data, interim biochemical assessments, and additional indicators of calcium metabolism, including parathyroid hormone, phosphate, ionized or albumin-adjusted calcium, and urinary calcium excretion, limits evaluation of calcium homeostasis and potential adverse effects. Consequently, the present study cannot establish the long-term safety or optimality of the prescribed supplementation regimen.

From a clinical perspective, these findings highlight the importance of considering seasonal variation, individual vitamin D status, and the potential risks of high-dose supplementation when managing endurance athletes. Supplementation decisions and biochemical monitoring should be guided by individual baseline micronutrient status, clinical circumstances, and potential safety concerns, particularly when vitamin D3 doses exceed established upper intake levels. Although substantial changes in circulating 25(OH)D and calcium were observed, the present study does not establish supplementation efficacy, optimal dosing, long-term safety, or improvements in athletic performance.

4.4. Vitamin B12 and Magnesium Status

Vitamin B12 and magnesium concentrations increased significantly over the five-month competitive season, with adjusted mean increases of 198.32 pg/mL (95% CI: 179.16–217.48) and 0.086 mmol/L (95% CI: 0.075–0.096), respectively (both q < 0.001). Neither the main effect of group nor the time × group interaction was statistically significant after FDR correction, although this does not establish equivalent responses between runners and triathletes. Vitamin B12 supports erythropoiesis and neurological function, whereas magnesium contributes to energy metabolism and neuromuscular activity. Nevertheless, increases in circulating concentrations should not automatically be interpreted as improvements in functional nutrient status, recovery, or athletic performance.

The observed increase in vitamin B12 can be considered in relation to findings reported by Krzywański et al. [11], who retrospectively analyzed 1131 blood samples from 243 elite track-and-field athletes. Vitamin B12 concentrations were higher in endurance athletes than in those representing strength-oriented disciplines (881 ± 32 vs. 703 ± 15 pg/mL; $p < 0.001$). The authors also reported higher circulating concentrations associated with vitamin B12 injections and a positive, but relatively weak, association with selected hematological parameters. Notably, the relationship with hemoglobin appeared to plateau at higher vitamin B12 concentrations, suggesting that further increases may not provide additional hematological

benefits. Differences in study design, participant characteristics, and supplementation route limit direct comparison with our cohort receiving oral vitamin B12.

More recent evidence was provided by Martínez–Noguera et al. [27], who conducted a randomized, placebo-controlled crossover trial involving 18 amateur male cyclists. Following three days of methylcobalamin supplementation (1000 µg/day), circulating vitamin B12 concentrations were higher than under placebo (924 ± 378 vs. 804 ± 343 pg/mL; $p = 0.008$), accompanied by improvements in selected anaerobic and cognitive outcomes. However, the small sample size, short intervention period, and specific functional assessments limit generalizability. In comparison, our participants received 500 µg/day over five months as part of a broader supplementation regimen. Because functional vitamin B12 status, hematological responses, and exercise performance were not assessed longitudinally in our study, the observed increase in serum vitamin B12 cannot establish improvements in these outcomes.

Magnesium-related biomarker responses may vary depending on training demands and supplementation conditions. Córdova et al. [18] investigated 18 professional male cyclists during a demanding 21-day stage race, comparing athletes receiving 400 mg/day of elemental magnesium with non-supplemented participants. Serum and erythrocyte magnesium concentrations decreased in both groups, although the reductions were attenuated by supplementation. Supplemented cyclists also exhibited a smaller increase in circulating myoglobin, suggesting a potentially different muscle-damage response. These findings contrast with the increase in serum magnesium observed in our cohort. However, the greater competitive demands, shorter observation period, higher supplementation dose (400 vs. 100 mg/day), and assessment of erythrocyte magnesium limit direct comparability. Together, these differences highlight the importance of training conditions, supplementation protocols, and biomarker selection when interpreting magnesium responses.

Additional evidence comes from Bomar et al. [28], who conducted a randomized, double-blind, placebo-controlled crossover trial involving 15 recreationally active adults. Eight days of magnesium chloride supplementation increased ionized magnesium concentrations from 0.47 ± 0.20 to 0.63 ± 0.09 mmol/L ($p = 0.015$). However, this biochemical increase was not accompanied by improved exercise performance; maximal oxygen uptake and mean sprint power were lower under the magnesium condition, while 10 km time-trial performance did not improve significantly. These findings demonstrate that higher circulating magnesium concentrations do not necessarily translate into functional benefits. Importantly, Bomar et al. assessed ionized magnesium, whereas our study measured total serum magnesium. Differences in biomarker selection, participant characteristics, and intervention duration therefore limit direct comparison between the studies.

Interpretation of these biomarker changes requires consideration of their physiological limitations. Serum magnesium represents only a small fraction of total-body magnesium and may not adequately reflect intracellular availability or tissue stores. Similarly, increased serum vitamin B12 does not necessarily indicate improved functional cobalamin status. The absence of erythrocyte or ionized magnesium measurements and complementary vitamin B12 markers, such as methylmalonic acid and homocysteine, limits a more comprehensive assessment of micronutrient status. Furthermore, dietary intake and training-related factors may have contributed to the observed changes, but their relative contributions cannot be determined from the available data.

From a practical perspective, these findings highlight the importance of interpreting vitamin B12 and magnesium concentrations in the context of individual nutritional status, dietary habits, training demands, and clinical symptoms. Biochemical monitoring and supplementation decisions should be individualized rather than based solely on increases in circulating biomarker concentrations. Given the single-arm, uncontrolled design, the

observed longitudinal changes cannot be attributed specifically to supplementation or interpreted as evidence of improved physiological function or athletic performance.

4.5. Metabolic and Biochemical Changes During the Competitive Season

During the five-month competitive season, significant reductions were observed in fasting glucose, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), non-high-density lipoprotein cholesterol (non-HDL-C), triglycerides (TG), ALT, and GGT, alongside an increase in high-density lipoprotein cholesterol (HDL-C) (all $q < 0.001$). Serum creatinine remained statistically unchanged. Neither the main group effects nor the time × group interactions remained significant after FDR correction, although this does not establish equivalent responses between runners and triathletes. These findings describe concurrent longitudinal biochemical changes without establishing their underlying causes or clinical consequences.

The observed lipid profile changes are consistent in direction with those reported in controlled exercise-training research. In a systematic review and meta-analysis of 148 randomized controlled trials involving 8673 participants, Smart et al. [29] reported significant reductions in TC (−5.90 mg/dL), LDL-C (−7.22 mg/dL), and TG (−8.01 mg/dL), alongside an increase in HDL-C (+2.11 mg/dL). Although the direction of these changes was consistent with our findings, the pooled effects cannot be directly compared with our longitudinal estimates. The meta-analysis included heterogeneous populations undergoing controlled exercise interventions, whereas our study followed already-trained endurance athletes without experimentally modifying their training programs. Consequently, the observed similarities do not establish that endurance training accounted for the lipid changes in our cohort, particularly given the potential influence of dietary and seasonal factors.

Importantly, favorable lipid changes are not consistently observed across longitudinal studies of competitive athletes. Di Gioia et al. [30], in a retrospective analysis of 717 Olympic athletes, reported no significant overall longitudinal changes in LDL-C, HDL-C, or TG. However, athletes with elevated baseline LDL-C showed a reduction of approximately 8.6%, suggesting that initial metabolic status may influence lipid trajectories. Similarly, Grzebisz–Zatońska et al. [31] observed reductions in fat mass and body fat percentage without significant changes in lipid parameters among 16 male cross-country skiers during a four-month training period. This demonstrates that body-composition changes and lipid responses do not necessarily occur in parallel. Furthermore, Ben Mhamed et al. [32] reported heterogeneous LDL-C and HDL-C responses over five months of endurance training, including significant group × time interactions between runners of different competitive levels and sedentary controls. Together, these findings suggest that baseline metabolic characteristics, training exposure, and competitive level may contribute to differences in lipid trajectories across studies. Such heterogeneity limits direct generalization to our cohort and cautions against attributing the observed lipid changes to a single factor.

Additional evidence of this variability was provided by Bizjak et al. [22], who assessed recreational endurance athletes following an intensive one-week training camp. Unlike our cohort, these athletes exhibited significant increases in TC, LDL-C, and HDL-C, whereas TG remained unchanged, despite reductions in fat mass and body fat percentage. These findings illustrate that favorable body-composition changes do not necessarily coincide with favorable lipid responses. However, the markedly shorter observation period and intensified training exposure limit direct comparisons with our five-month study and suggest that short-term exercise-related biochemical fluctuations should be distinguished from longer-term seasonal trajectories.

The significant reduction in fasting glucose observed in our study (−1.74 mg/dL; $q < 0.001$) may also be considered in the context of training-related metabolic regulation.

Delvecchio et al. [33] examined 27 male masters cyclists across different 12-week training conditions and found no significant overall change in fasting glucose ($p = 0.35$), despite a numerical reduction among athletes combining endurance and sprint training. Conversely, Liao et al. [34] reported that eight weeks of training cessation in 16 elite taekwondo athletes were associated with increases in fasting glucose (9.84%; $p = 0.004$) and insulin resistance assessed using HOMA-IR (34.2%; $p = 0.006$). Although these studies involved different athletic populations and training conditions, they highlight the variability of glycemic responses across training contexts. Importantly, the modest reduction in fasting glucose observed in our cohort cannot be interpreted as evidence of improved insulin sensitivity or metabolic flexibility, as fasting insulin, HOMA-IR, and dynamic measures of glucose metabolism were not assessed.

ALT and GGT activities decreased significantly during follow-up, with adjusted mean changes of −3.66 and −2.24 U/L, respectively (both $q < 0.001$). However, these enzymes are not direct measures of hepatic function, and ALT activity may also be influenced by skeletal muscle-related processes. Devries et al. [35] examined 12 weeks of endurance training in previously inactive adults and reported a significant reduction in GGT among men ($p = 0.01$), whereas ALT and hepatic lipid content did not change significantly. Their findings are consistent with the GGT reduction observed in our cohort but differ regarding ALT, which also decreased in our participants. Importantly, these observations indicate that changes in circulating liver-related enzymes should not automatically be interpreted as improvements in hepatic function or reductions in liver fat.

Other investigations have demonstrated substantially different liver-related biochemical responses under more demanding exercise conditions. Córdova et al. [18] reported increased ALT activity in professional cyclists during a 21-day stage race, irrespective of magnesium supplementation. Similarly, Bizjak et al. [22] observed increased ALT activity following an intensive one-week endurance training camp, whereas GGT remained unchanged. In contrast, Manechini et al. [21] reported no significant longitudinal changes in ALT during a 12-week marathon training program. Together, these findings suggest that exercise intensity, recovery conditions, and blood-sampling timing may contribute to divergent enzyme trajectories, limiting direct comparisons with the decreases observed in our five-month cohort.

Serum creatinine remained statistically unchanged during the observation period (adjusted mean change: −0.002 mg/dL; $q = 0.588$). This result is consistent with findings reported by Banfi et al. [36], who observed no significant seasonal change in serum creatinine among elite cyclists ($p = 0.25$), although responses differed across sporting disciplines. In contrast, Bizjak et al. [22] reported increased creatinine concentrations following an intensive one-week endurance training camp. Differences in exercise exposure, muscle metabolism, hydration, and sampling conditions may contribute to these divergent findings. Nevertheless, stable serum creatinine in our cohort does not establish unchanged renal function, particularly because additional renal biomarkers and direct measures of glomerular filtration were unavailable.

Overall, these findings highlight the importance of interpreting metabolic and biochemical markers collectively when evaluating endurance athletes during a competitive season. However, the single-arm design, absence of a non-supplemented control group, and limited information on dietary intake and training intensity prevent attribution of the observed changes specifically to supplementation or endurance training. Furthermore, without complementary metabolic and physiological assessments, reductions in glucose, lipid concentrations, and liver-related enzymes cannot be interpreted as evidence of improved insulin sensitivity, hepatic function, or cardiovascular outcomes. Rather, the findings

provide an integrated description of longitudinal biochemical changes occurring during the investigator-prescribed supplementation intervention.

4.6. Strengths and Limitations

The present study has several strengths. Its prospective design enabled the assessment of longitudinal changes in micronutrient-related biomarkers, body composition, and metabolic parameters over a five-month competitive season. The use of a standardized, investigator-prescribed supplementation regimen and consistent assessment procedures provided a relatively uniform framework for evaluating these changes. Moreover, the simultaneous assessment of multiple physiological domains allowed a broader characterization of biochemical and body-composition trajectories than investigations focusing on individual micronutrients. The application of LMMs accounted for repeated measurements within participants and enabled adjustment for selected potential confounders, while FDR correction addressed the risk of false-positive findings arising from multiple testing.

Several limitations should nevertheless be acknowledged. First, the single-arm design without a non-supplemented control group precludes causal attribution of the observed changes to supplementation. The effects of the prescribed regimen cannot be distinguished from those of habitual training, seasonal variation, dietary intake, or other unmeasured influences. Second, the modest sample size ($n = 50$), including 25 athletes per discipline, may have limited the ability to detect smaller between-group differences and time × group interactions. The inclusion of male athletes only further restricts generalizability. Third, the study covered a single competitive season with only baseline and follow-up measurements, preventing characterization of intermediate fluctuations and the persistence of observed changes.

Additional limitations concern exposure assessment and measurement specificity. Detailed dietary intake, individual sunlight exposure, training intensity, and periodization were not comprehensively documented, while training exposure was primarily characterized by reported weekly training hours. Body-composition estimates obtained using bioelectrical impedance analysis may also be influenced by hydration-related variability despite standardized measurement conditions.

Another limitation concerns the timing of follow-up blood sampling. Although all follow-up samples were collected within seven days after completion of the supplementation period, the exact timing varied among participants according to their availability. Consequently, the interval between the last supplement intake and blood collection was not standardized. This variability may have influenced the measured biochemical concentrations, particularly serum iron, which is sensitive to recent oral iron intake, and should be considered when interpreting the observed longitudinal changes.

Furthermore, the absence of hepcidin, inflammatory markers, complementary iron indices, functional vitamin B12 markers, and additional measures of calcium and magnesium homeostasis limits interpretation of the underlying physiological mechanisms. The lack of interim biochemical assessments and comprehensive safety-related measurements also restricts conclusions regarding the safety of the prescribed supplementation regimen. Finally, the absence of longitudinal measures of insulin sensitivity, hepatic function, and athletic performance prevents assessment of whether the observed biochemical changes translated into clinically or functionally meaningful benefits.

Despite these limitations, the study provides an integrated longitudinal description of biochemical and body-composition changes in male endurance athletes receiving an investigator-prescribed micronutrient regimen while maintaining their usual training practices. These findings may inform future adequately powered, controlled studies incorporat-

ing detailed exposure assessment, repeated biochemical monitoring, and clinically relevant functional outcomes.

4.7. Practical Implications

The present findings highlight the importance of interpreting micronutrient-related and metabolic biomarkers in the context of athletes' baseline nutritional status, dietary habits, training demands, and seasonal exposure. When clinically indicated, longitudinal biochemical assessment may help identify changes requiring further evaluation and support individualized nutritional management. However, circulating biomarker concentrations alone do not establish optimal nutrient status or functional benefits. Supplementation decisions should therefore consider individual risk factors, documented deficiencies, and potential safety concerns, particularly when higher vitamin D doses are prescribed alongside calcium.

Importantly, the single-arm, uncontrolled design prevents attribution of the observed changes specifically to supplementation and does not support recommendations for routine micronutrient supplementation or particular dosing regimens in endurance athletes. Further controlled studies incorporating detailed dietary and training assessments, supplementation adherence, safety monitoring, and functional outcomes are needed to clarify the clinical relevance of these longitudinal changes and inform evidence-based supplementation strategies.

5. Conclusions

This prospective, single-arm, uncontrolled supplementation intervention documented significant longitudinal changes in micronutrient-related biomarkers, body composition, and selected metabolic parameters in male runners and triathletes over a five-month competitive season. The study adds longitudinal data on concurrent changes in micronutrient-related biomarkers, body composition, and metabolic parameters in male runners and triathletes following a common physician-prescribed supplementation regimen across a competitive season. No time × group interactions remained significant after FDR correction, although this does not establish equivalent physiological responses between runners and triathletes.

From a practical perspective, the observed longitudinal changes in iron-related and micronutrient biomarkers highlight the potential value of follow-up biochemical assessment in endurance athletes undergoing physician-prescribed supplementation. Evaluating these changes alongside body-composition and metabolic parameters may provide a more comprehensive picture of athletes' physiological status throughout a competitive season and support individualized clinical monitoring.

Importantly, the absence of a non-supplemented control group prevents causal attribution of these changes to the investigator-prescribed supplementation regimen. Seasonal variation, habitual training, dietary intake, and other unmeasured factors may also have contributed to the observed trajectories. Furthermore, biochemical changes alone do not demonstrate improved nutritional adequacy, physiological function, or athletic performance. The findings therefore support the value of an integrated approach to evaluating longitudinal biomarker changes in endurance athletes but do not establish supplementation efficacy, optimal dosing, or long-term safety. Future controlled studies incorporating detailed exposure assessment, safety monitoring, and functional outcomes are needed to clarify the clinical significance of these observations.

Author Contributions: Conceptualization, M.G.; methodology, P.V.C. and M.G.; software, P.V.C.; validation, P.V.C. and M.G.; formal analysis, P.V.C.; investigation, M.G.; data curation, M.G.; writing—original draft preparation, P.V.C.; writing—review and editing, P.V.C. and M.G.; visualization, P.V.C.; supervision, M.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Toruń (protocol code KB 407/2023 and date of approval: 26 September 2023).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author due to privacy and ethical restrictions.

Acknowledgments: The authors would like to acknowledge Servier Medical Art for providing the vector elements used in the graphical abstract, licensed under a Creative Commons Attribution 4.0 International License (CC BY 4.0).

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

25(OH)D	25-Hydroxyvitamin D
ALT	Alanine aminotransferase
ATP	Adenosine triphosphate
BIA	Bioelectrical impedance analysis
BM	Body mass
BMI	Body mass index
Ca	Calcium
CI	Confidence Interval
ECW	Extracellular water
ECW/TBW	Extracellular-to-total body water ratio
ECLIA	Electrochemiluminescence immunoassay
FDR	False discovery rate
FFM	Fat-free mass
FM	Fat mass
GGT	Gamma-glutamyl transferase
HDL-C	High-density lipoprotein cholesterol
LDL-C	Low-density lipoprotein cholesterol
LMM	Linear mixed-effects models
Mg	Magnesium
MM	Skeletal muscle mass
non-HDL-C	Non-high-density lipoprotein cholesterol
REML	Restricted maximum likelihood
SD	Standard deviation
T0	Baseline assessment
T1	Follow-up assessment
TBW	Total body water
TC	Total cholesterol
TG	Triglycerides
VAT	Visceral adipose tissue
VIF	Variance inflation factor

References

1. Thomas, D.T.; Erdman, K.A.; Burke, L.M. Position of the Academy of Nutrition and Dietetics, Dietitians of Canada, and the American College of Sports Medicine: Nutrition and athletic performance. *J. Acad. Nutr. Diet.* **2016**, *116*, 501–528. [CrossRef] [PubMed]
2. Vitale, K.; Getzin, A. Nutrition and Supplement Update for the Endurance Athlete: Review and Recommendations. *Nutrients* **2019**, *11*, 1289. [CrossRef] [PubMed]

3. Beck, K.L.; von Hurst, P.R.; O'Brien, W.J.; Badenhorst, C.E. Micronutrients and Athletic Performance: A Review. *Food Chem. Toxicol.* **2021**, *158*, 112618. [CrossRef] [PubMed]
4. Moss, K.; Kreutzer, A.; Graybeal, A.J.; Zhang, Y.; Braun-Trocchio, R.; Porter, R.R.; Shah, M. Nutrient Adequacy in Endurance Athletes. *Int. J. Environ. Res. Public Health* **2023**, *20*, 5469. [CrossRef] [PubMed]
5. Sim, M.; Garvican-Lewis, L.A.; Cox, G.R.; Govus, A.; McKay, A.K.A.; Stellingwerff, T.; Peeling, P. Iron Considerations for the Athlete: A Narrative Review. *Eur. J. Appl. Physiol.* **2019**, *119*, 1463–1478. [CrossRef] [PubMed]
6. Solberg, A.; Reikvam, H. Iron Status and Physical Performance in Athletes. *Life* **2023**, *13*, 2007. [CrossRef] [PubMed]
7. Pengelly, M.; Pumpa, K.; Pyne, D.B.; Etxebarria, N. Iron Deficiency, Supplementation, and Sports Performance in Female Athletes: A Systematic Review. *J. Sport Health Sci.* **2025**, *14*, 101009. [CrossRef] [PubMed]
8. Larsuphrom, P.; Latunde-Dada, G.O. Association of Serum Hepcidin Levels with Aerobic and Resistance Exercise: A Systematic Review. *Nutrients* **2021**, *13*, 393. [CrossRef] [PubMed]
9. Šmid, A.N.; Golja, P.; Hadžić, V.; Abazović, E.; Drole, K.; Paravlic, A.H. Effects of Oral Iron Supplementation on Blood Iron Status in Athletes: A Systematic Review, Meta-Analysis and Meta-Regression of Randomized Controlled Trials. *Sports Med.* **2024**, *54*, 1231–1247. [CrossRef] [PubMed]
10. Wyatt, P.B.; Reiter, C.R.; Satalich, J.R.; O'Neill, C.N.; Edge, C.; Cyrus, J.W.; O'Connell, R.S.; Vap, A.R. Effects of Vitamin D Supplementation in Elite Athletes: A Systematic Review. *Orthop. J. Sports Med.* **2024**, *12*, 23259671231220371. [CrossRef] [PubMed]
11. Krzywański, J.; Mikulski, T.; Pokrywka, A.; Młyńczak, M.; Krysztofiak, H.; Frączek, B.; Ziemba, A. Vitamin B12 Status and Optimal Range for Hemoglobin Formation in Elite Athletes. *Nutrients* **2020**, *12*, 1038. [CrossRef] [PubMed]
12. Tarsitano, M.G.; Quinzi, F.; Folino, K.; Greco, F.; Oranges, F.P.; Cerulli, C.; Emerenziani, G.P. Effects of Magnesium Supplementation on Muscle Soreness in Different Type of Physical Activities: A Systematic Review. *J. Transl. Med.* **2024**, *22*, 629. [CrossRef] [PubMed]
13. Bosy-Westphal, A.; Jensen, B.; Braun, W.; Pourhassan, M.; Gallagher, D.; Müller, M.J. Quantification of Whole-Body and Segmental Skeletal Muscle Mass Using Phase-Sensitive 8-Electrode Medical Bioelectrical Impedance Devices. *Eur. J. Clin. Nutr.* **2017**, *71*, 1061–1067. [CrossRef] [PubMed]
14. Kyle, U.G.; Bosaeus, I.; De Lorenzo, A.D.; Deurenberg, P.; Elia, M.; Gómez, J.M.; Heitmann, B.L.; Kent-Smith, L.; Melchior, J.-C.; Pirlich, M.; et al. Bioelectrical Impedance Analysis—Part II: Utilization in Clinical Practice. *Clin. Nutr.* **2004**, *23*, 1430–1453. [CrossRef] [PubMed]
15. Płudowski, P.; Kos-Kudła, B.; Walczak, M.; Fal, A.; Zozulińska-Ziółkiewicz, D.; Sieroszewski, P.; Peregud-Pogorzelski, J.; Lauterbach, R.; Targowski, T.; Lewiński, A.; et al. Guidelines for Preventing and Treating Vitamin D Deficiency: A 2023 Update in Poland. *Nutrients* **2023**, *15*, 695. [CrossRef] [PubMed]
16. McCormick, R.; Dreyer, A.; Dawson, B.; Sim, M.; Lester, L.; Goodman, C.; Peeling, P. The Effectiveness of Daily and Alternate Day Oral Iron Supplementation in Athletes with Suboptimal Iron Status (Part 2). *Int. J. Sport Nutr. Exerc. Metab.* **2020**, *30*, 191–196. [CrossRef] [PubMed]
17. Gervasi, M.; Fernández-Peña, E.; Zeppa, S.D.; Annibalini, G.; Bartolacci, A.; Formiglio, E.; Agostini, D.; Barbato, C.; Fiaccarini, G.; Spaccazocchi, I.; et al. Effects of Vitamin D Supplementation during Autumn and Winter on Blood Biomarkers and Physical Performance in Runners and Non Runners. *Sci. Rep.* **2026**, *16*, 10094. [CrossRef] [PubMed]
18. Córdova, A.; Mielgo-Ayuso, J.; Roche, E.; Caballero-García, A.; Fernández-Lázaro, D. Impact of Magnesium Supplementation in Muscle Damage of Professional Cyclists Competing in a Stage Race. *Nutrients* **2019**, *11*, 1927. [CrossRef] [PubMed]
19. Córdova, A.; Mielgo-Ayuso, J.; Fernandez-Lazaro, C.I.; Caballero-García, A.; Roche, E.; Fernández-Lázaro, D. Effect of Iron Supplementation on the Modulation of Iron Metabolism, Muscle Damage Biomarkers and Cortisol in Professional Cyclists. *Nutrients* **2019**, *11*, 500. [CrossRef] [PubMed]
20. Ishibashi, A.; Maeda, N.; Sumi, D.; Goto, K. Elevated Serum Hepcidin Levels during an Intensified Training Period in Well-Trained Female Long-Distance Runners. *Nutrients* **2017**, *9*, 277. [CrossRef] [PubMed]
21. Manechini, J.P.V.; Aquino, R.; Moraes, C.; Tourinho Filho, H.; Pimenta, P.M.; Puggina, E.F. Long Distance Training Associated to HIIT Protocol Does Not Induce Changes in Blood Biochemical Markers in Adult Marathoners. *Rev. Bras. Ciênc. Esporte* **2020**, *42*, e2032. [CrossRef]
22. Bizjak, D.A.; John, L.; Munk, M.; Reiter, M.; Lüders, N.; Kirsten, J.; Henze, A.-S.; Schulz, S.V.W. Physiological, Metabolic, and Mitochondrial Adaptations to a One-Week Endurance Training Camp in Recreational Athletes: An Observational Study. *Sports* **2026**, *14*, 200. [CrossRef] [PubMed]
23. Krzywanski, J.; Mikulski, T.; Krysztofiak, H.; Mlynczak, M.; Gaczynska, E.; Ziemba, A. Seasonal Vitamin D Status in Polish Elite Athletes in Relation to Sun Exposure and Oral Supplementation. *PLoS ONE* **2016**, *11*, e0164395. [CrossRef] [PubMed]
24. Wilson-Barnes, S.L.; Hunt, J.E.A.; Williams, E.L.; Allison, S.J.; Wild, J.J.; Wainwright, J.; Lanham-New, S.A.; Manders, R.J.F. Seasonal Variation in Vitamin D Status, Bone Health and Athletic Performance in Competitive University Student Athletes: A Longitudinal Study. *J. Nutr. Sci.* **2020**, *9*, e8. [CrossRef] [PubMed]

25. Kasprowicz, K.; Ratkowski, W.; Wołyniec, W.; Kaczmarczyk, M.; Witek, K.; Żmijewski, P.; Renke, M.; Jastrzębski, Z.; Rosemann, T.; Nikolaidis, P.T.; et al. The Effect of Vitamin D3 Supplementation on Hepcidin, Iron, and IL-6 Responses after a 100 km Ultra-Marathon. *Int. J. Environ. Res. Public Health* **2020**, *17*, 2962. [CrossRef] [PubMed]
26. EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens); Turck, D.; Bohn, T.; Castenmiller, J.; de Henauw, S.; Hirsch-Ernst, K.I.; Knutsen, H.K.; Maciuk, A.; Mangelsdorf, I.; McArdle, H.J.; et al. Scientific Opinion on the Tolerable Upper Intake Level for Vitamin D, Including the Derivation of a Conversion Factor for Calcidiol Monohydrate. *EFSA J.* **2023**, *21*, e08145. [CrossRef] [PubMed]
27. Martínez-Noguera, F.J.; Alcaraz, P.E.; González Blanc, F.J.; Huyghe, T.G.; Marín-Pagán, C. Short-Term Methylcobalamin Supplementation Is Associated with Changes in Anaerobic and Cognitive Performance in Amateur Cyclists: A Randomized Crossover Trial. *Nutraceuticals* **2026**, *6*, 35. [CrossRef]
28. Bomar, M.C.; Ewell, T.R.; Brown, R.L.; Brown, D.M.; Kwarteng, B.S.; Abbotts, K.S.S.; Butterklee, H.M.; Williams, N.N.B.; Wrigley, S.D.; Walsh, M.A.; et al. Short-Term Magnesium Supplementation Has Modest Detrimental Effects on Cycle Ergometer Exercise Performance and Skeletal Muscle Mitochondria and Negligible Effects on the Gut Microbiota: A Randomized Crossover Clinical Trial. *Nutrients* **2025**, *17*, 915. [CrossRef] [PubMed]
29. Smart, N.A.; Downes, D.; van der Touw, T.; Hada, S.; Dieberg, G.; Pearson, M.J.; Wolden, M.; King, N.; Goodman, S.P.J. The Effect of Exercise Training on Blood Lipids: A Systematic Review and Meta-Analysis. *Sports Med.* **2025**, *55*, 67–78. [CrossRef] [PubMed]
30. Di Gioia, G.; Buzzelli, L.; Maestrini, V.; Squeo, M.R.; Lemme, E.; Monosilio, S.; Serdoz, A.; Fiore, R.; Zampaglione, D.; Segreti, A.; et al. Long-Term Evaluation of Lipid Profile Changes in Olympic Athletes. *Int. J. Sport Nutr. Exerc. Metab.* **2024**, *34*, 267–274. [CrossRef] [PubMed]
31. Grzebisz-Zatońska, N.; Grzywacz, T.; Waśkiewicz, Z. The Influence of Endurance Training on the Lipid Profile, Body Mass Composition and Cardiovascular Efficiency in Middle-Aged Cross-Country Skiers. *Int. J. Environ. Res. Public Health* **2021**, *18*, 10928. [CrossRef] [PubMed]
32. Ben Mhamed, M.; Ben Maaouia, G.; Landolsi, M.; Eken, Ö.; Çalışır, M.; Aslan, T.V.; Aldhahi, M.I. Aerobic, Hematological, and Lipid Changes after Five Months of Endurance Training in Distance Runners. *Sci. Rep.* **2026**, *16*, 19797. [CrossRef] [PubMed]
33. Delvecchio, L.; Reaburn, P.; Trapp, G.; Korhonen, M.T. Effect of Concurrent Resistance and Sprint Training on Body Composition and Cardiometabolic Health Indicators in Masters Cyclists. *J. Exerc. Rehabil.* **2016**, *12*, 442–450. [CrossRef] [PubMed]
34. Liao, Y.-H.; Sung, Y.-C.; Chou, C.-C.; Chen, C.-Y. Eight-Week Training Cessation Suppresses Physiological Stress but Rapidly Impairs Health Metabolic Profiles and Aerobic Capacity in Elite Taekwondo Athletes. *PLoS ONE* **2016**, *11*, e0160167. [CrossRef] [PubMed]
35. Devries, M.C.; Samjoo, I.A.; Hamadeh, M.J.; Tarnopolsky, M.A. Effect of Endurance Exercise on Hepatic Lipid Content, Enzymes, and Adiposity in Men and Women. *Obesity* **2008**, *16*, 2281–2288. [CrossRef] [PubMed]
36. Banfi, G.; Del Fabbro, M.; Lippi, G. Creatinine Values during a Competitive Season in Elite Athletes Involved in Different Sport Disciplines. *J. Sports Med. Phys. Fit.* **2008**, *48*, 479–482.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.