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A comprehensive study to determine the relationship between functional supplements, sports gene, and biochemical indicators in athletes

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ABSTRACT

Today, various dietary supplements are taken to improve endurance performance during physical activity. These supplements have proven to be very successful in controlling and balancing various hormone levels and have contributed to the prevention and healing of metabolic disorders and physical injuries. In this study, we sought to investigate the effects of the *ACTN3* gene and nutrient supplementation, including beta-alanine, ashwagandha, L-carnitine, and iron, on athletic performance. The *ACTN3* gene has been identified as a potential genetic determinant of athletic performance, particularly in activities that require high levels of power and strength. This study investigated the interaction between the *ACTN3* gene and supplementation and highlighted the potential for personalized approaches to improve athletic performance. 24 subjects per group, were randomized in a parallel-group design to examine the effects of supplementation on the *ACTN3* gene and athletic performance over a 21-day intervention period. Participants in the intervention were divided into four groups of six members each. The same subjects who did not take supplements formed the control group. In addition, the subjects were divided into 4 groups (G1, G2, G3, and G4), each consisting of subgroups of control and a treated. All treated groups (TG1, TG2, TG3, and TG4) received 4 supplements for 21 days, namely S1 (ashwagandha), S2 (iron), S3 (beta-alanine) and S4 (L-carnitine), together with the 4 control groups, i.e. CG1, CG2, CG3 and CG4, who received no supplements during the same period. In particular, this study determines the effect of these 4 supplements on *ACTN3* gene expression, testosterone, cholesterol and BUN levels of the participants. The P-values determined for all parameters showed statistically significant values and the final result showed that all supplements had positive effects on the tested parameters. The result showed a highly significant difference between the intake of supplements before and after the intake of 24 athletes per group on the *ACTN3* gene expression level and biochemical indicators.

Keywords: *ACTN3* gene, Ashwagandha, Iron, Beta-alanine, L-carnitine, Testosterone, Biochemical indicators

INTRODUCTION

The interaction between nutrition and DNA to alter gene expression leads to our unique metabolic phenotype. Although physical training alters the human skeletal muscle epigenome and the resulting gene expression [1] .There are two types of muscle fibers in the human body, namely slow-twitch muscle fibers (type I muscle fibers) and fast-twitch muscle fibers (type II muscle fibers). All types of muscle fibers express the ACTN2 gene, but only type II fibers, especially type IIb fibers, express the *ACTN3* gene. The *ACTN3* gene produces a protein known as alpha-actinin-3, which is found in fast-twitch muscles that are critical for speed and strength activities such as sprinting and weightlifting. One gene that is linked to human physical performance is *ACTN3*, which codes for the protein actinin-3. This alpha-actinin-3 protein is a component of the sarcomeric apparatus in the fast

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glycolytic fibers of the human skeletal system, which are responsible for generating the powerful, rapid contractions that occur during exercise. It is thought to perform a specific task or role that is critical to the functionality of these fibers [2]. In humans, two genes encode α-actinins, which are found in skeletal muscle: ACTN2, which is expressed in all skeletal muscle fibers, and *ACTN3*, whose expression is restricted to type II fibers and which is primarily involved in strong, explosive contractions. The loss of alpha-actinin-3 is caused by the polymorphism of this gene *R577X* (rs1815739) [3]. A variation in this gene (*R577X*) has been linked to differences in muscle performance and athletic ability. A complex interplay of genetic and environmental factors influences athletic performance. Among the genetic factors, the *ACTN3* gene has received considerable attention as a potential determinant of athletic performance, particularly in activities requiring high levels of power and strength. In particular, there is a common variation known as the RR and RX alleles. The "R" allele is associated with the production of alpha-actinin-3 proteins, while fewer type II fibers are formed. The type II muscle fibers are responsible for fast and powerful movements. The "X" allele is associated with the absence of this protein. People who have inherited the RR allele are more likely to have high levels of alpha-actinin-3 protein in their muscles and may have an advantage in activities that require explosive power, such as sprinting and jumping. A person who has only one copy of the X gene with the RX allele, on the other hand, must exert themselves to be successful in these activities. In contrast, the X allele is associated with lower levels of alpha-actinin3 proteins in the muscles, which can reduce muscle strength and performance. People with the "RR" genotype, which is associated with functional *ACTN3* expression, may have a greater proportion of fast-twitch muscle fibers than people with the "RX" genotype. This genetic influence on the type of muscle fibers may result in differences in athletic performance [4] .The researchers' work shows that in animal models, the *ACTN3* knockout mouse with *ACTN3* deficiency has reduced activity in the anaerobic glycolytic metabolic pathway and increased activity in the aerobic oxidative metabolic pathway. It is noteworthy that in European Caucasian populations, homozygosity for an early stop codon polymorphism in the *ACTN3* gene causes a complete deficiency of α-actinin-3 protein in approximately 18 humans. However, this defect does not result in a disease phenotype or impaired muscle function. However, several research results indicate that the ability to perform strong muscle contractions is positively correlated with the presence of the R allele. However, the X allele may predispose to higher performance in endurance exercise. For this reason, Yang et al. discovered in a sample of elite white athletes that both male and female sprinters had a higher frequency of the *577R* gene[2,5,6]. Only fast-twitch muscle fibers (type IIb/x fibers in 100% and type IIa fibers in 50%) express *ACTN3*. In the Z-membrane, alpha-actinins play a crucial structural role by establishing the cross-connection between the thin actin filaments [2]. They play a regulatory role in the regulation of myofiber contraction as well as a static role in the maintenance of ordered myofibril arrangements [2].

Nutrient supplementation and athletic performance

Nutrient supplementation is of interest as a possible tactic to improve athletic performance. The potential effects of some nutrients such as ashwagandha, iron, beta-alanine, and L-carnitine have been studied.

Ashwagandha: Ashwagandha (*Withania somnifera*, Solanaceae) Also known as "Indian winter cherry" or "Indian ginseng". It promotes both physical and mental health and has been consumed as a tonic for many years. Ashwagandha is excellent for improving the function of the nervous and brain systems. As it also promotes sexual health, it is excellent for improving reproductive balance. Ashwagandha also improves cell-mediated immunity, which leads to a strengthening of the body's defenses against various diseases [1,7].

Sitoindosides and acylsterylglucosides are two of the many bioactive compounds in ashwagandha that are particularly effective as anti-stress agents [8]. Ashwagandha isolates Withaferin A and 3b Hydroxy 2 and Dihydro Withanolide F have promising antitumor, immunomodulatory, antibacterial and anti-inflammatory properties [9]. It proved to be effective against 18-hour immobilization, cold and aspirin-induced gastrointestinal ulcers due to its anti-ulcerogenic effect [9] Ribosome 35S incorporation in granulation tissue is significantly inhibited by ashwagandha. The mitochondria of granulation tissue were also affected by the uncoupling effect (reduction of ADP/O ratio) and Mg^{2+}-dependent ATPase activity was impaired by ashwagandha [10]. It can also help with muscle repair and minimize the damage caused by exercise-induced muscle injuries.

Iron: Iron is an important component of haemoglobin, which is contained in red blood cells. It has been proven that taking an iron supplement increases haemoglobin levels, improves oxygen transport, and possibly increases

endurance [11]. The main functions of the important mineral iron are the reversible transportation of oxygen in the hemoglobin molecules of red blood cells and the myoglobin of muscle cells. It also plays a crucial role in the formation of DNA, enzymes, energy, and the electron transport chain. Metabolism [12,13]. Cells take up iron and transport it to the mitochondria, where it is converted to heme or porphyrin-bound iron, the form of iron found in myoglobin and hemoglobin molecules. Since iron ions can send and receive electrons, their chemistry is important for biological processes. By acting as a cofactor for many proteins and enzymes, it can accelerate redox processes [14]. Due to its redox potential, iron can also be harmful in high concentrations as it can produce Reactive Oxygen Species (ROS), which can be harmful or even lead to death [15]. Iron is transported through the blood by the transport protein transferrin and additional iron is stored as ferritin in the liver and in the reticuloendothelial system to prevent unfavourable reactions [12,15]. The most used biomarker for iron status is ferritin [16]. Haemoglobin, myoglobin, and enzymes actively consume most of the iron in the body. Men and women store about 4 g and 2.5 g of iron respectively, but due to iron absorption in the intestine and an effective iron recycling mechanism, only 1-2 mg of iron is lost daily [12,16] .The average daily iron consumption is between 10 mg and 15 mg, but only under normal circumstances, 10% is absorbed, while iron losses due to epithelial desquamation and slight bleeding only occur in traces [16].

The peptide hormone hepcidin, which is produced by the hepatocytes in the liver, precisely controls the iron balance in the blood [17]. The need for iron for erythropoiesis controls the upregulation of hepcidin, which occurs when serum ferritin levels are high. By attaching to its receptor on the ferroportin, which transports Fe^{2+} from the enterocytes into the plasma, hepcidin prevents the absorption of iron [16,17]. It has also been shown that inflammatory indicators such as Interleukin-6 (IL-6) stimulate the formation of hepcidin. Athletes with lower baseline ferritin levels showed a lower hepcidin response after exercise, suggesting that this response is also influenced by pre-exercise ferritin levels [9,10]. Current research focuses on a possible altered hepcidin response associated with a low-carbohydrate or ketogenic diet and Low Energy Availability (LEA) [18-22].

Beta-alanine: Beta-alanine is an amino acid that serves as a building block for the skeletal muscle production of carnosine. Carnosine is a cytoplasmic dipeptide found in significant amounts in the skeletal muscles of monkeys and invertebrates as well as in the central nervous system. It is now known that carnosine synthase, which is mainly found in muscle and brain tissue, catalyzes the reaction between histidine and beta-alanine to produce carnosine. Since the blood contains the enzyme carnosinase, carnosine is rapidly hydrolyzed to produce amino acids [23,24]. Alanine and histidine can then reach various tissues and organs. By regulating pH and buffering during high-intensity exercise, carnosine can delay the onset of fatigue. Supplementation with beta-alanine has been associated with higher muscle carnosine levels and improved athletic performance [25,26].

L-carnitine: L-carnitine (3-hydroxy-4-N-trimethylammo niobutanoate) is a conditionally essential amino acid used in energy production and fatty acid metabolism. Carnitine is essential for health and helps with energy synthesis and fatty acid metabolism. Compared to meat eaters, vegetarians have a higher bioavailability. If it is not ingested with food, the amino acids methionine and lysine are two important building blocks are converted to creatine in the body. This can take place in the kidneys, liver, and brain [27].

The largest amounts are found in the skeletal and heart muscles, which cannot synthesize carnitine and must instead obtain it from the plasma. 99% of carnitine is found in the cells [27]. Carnitine influences the metabolism of carbohydrates. Diabetes, trauma, hemodialysis, starvation, obesity, cardiomyopathy, fasting, endocrine imbalances, drug interactions, and other disorders have been associated with abnormalities in the regulation of carnitine [28]. Carnitine counteracts muscle atrophy. Muscle wasting or atrophy is a reduction in muscle mass in various chronic diseases, including infectious diseases and cancer, which affects the quality of life because muscle mass is essential for the body's overall metabolism [29-31] .Results from animal research and clinical studies show that L-carnitine supplementation has a positive effect on many critical signaling pathways involved in pathological skeletal muscle breakdown, improved nitrogen balance through increased protein synthesis or decreased protein degradation, prevention of apoptosis and abrogation of inflammatory responses under pathological conditions [32]. It has been demonstrated that the induced transcription factor Nuclear Factor-kB (NF-kB) is a transcriptional regulator of atrogin-1 and MuRF1. In animal models, constitutive activation of NF-kB and cytokines or Reactive Oxygen Species (ROS) increased the expression of atrogin-1 and MuRF1 in skeletal muscle, leading to atrophy and muscle loss [33]. On the other hand, inhibition

of NF-kB activation of skeletal muscle stops the signaling of inflammation and atrophy, significantly preserving muscle mass in response to systemic inflammation [34].

Testosterone: The anabolic steroid hormone testosterone occurs in three forms in the bloodstream: Free (~0.5%-3%), weakly bound (~30%) to albumin, and tightly bound (~70%) to Sex Hormone-Binding Globulin (SHBG). Historically, the maintenance and growth of skeletal muscle mass (hypertrophy) has been the physiological role of testosterone in skeletal muscle tissue [35]. It is believed that α-Actinin-3 (*ACTN3*) controls skeletal muscle development and hypertrophy through interaction with the signaling protein calcineurin. Research shows that serum testosterone levels can be affected by α-actinin-3 insufficiency, which is predicted by the *ACTN3* XX genotype, as inhibition of calcineurin increases the production of testosterone [36]. It has been observed that an increase in the number of myonuclei and cross-sectional area of both type I and type II fiber types contributes to testosterone-induced increases in muscle size and strength. Research suggests that the positive effects of testosterone on muscle protein synthesis are responsible for its positive effects on skeletal muscle shape [36-38].Despite the increase in testosterone in the blood, androgen signaling in the neurons of the brain is required to maintain muscle hypertrophy in fast-twitch muscles.

By stimulating the Wnt-κ-catenin pathway, genomic androgen/androgen receptor binding can improve muscle function [39]. When Wnt binds to the Frizzled/ lipoprotein receptor protein 6 receptors, it activates Dishevelled (Dsh) and inhibits Glycogen Synthase Kinase (GSK-3), which decreases the activity of α-catenin and promotes its dephosphorylation [40].The Fluorotoluene Androgen Receptor FT-AR complex binds to DNA response elements (T cell factor/lymphoid enhancer factor 1-TCF/LEF), inhibits GSK-3, and increases α-catenin, which then translocates to the nucleus, boosts transcription and activates muscle satellite cells. Since α-catenin has no nuclear localization sequence, it requires cytosolic proteins that have a sequence that aids in translocation. The androgen receptor complex can help α-catenin to enter the nucleus and attach to DNA regions there [41]. Testosterone increases transcriptional capacity and androgen receptor-α-catenin interaction. Positive correlations have been observed between Wnt5 expression and the amount of androgen receptor protein. Treatment with testosterone increases the expression of Wnt5 protein in muscles in a dose-dependent manner [39-42].

MATERIALS AND METHODS

The present study included 24 subjects per group, who were randomized into parallel groups to investigate the effects of dietary supplementation on *ACTN3* gene, testosterone levels, athletic performance, cholesterol and Bun levels, during a 21-day intervention period. Mainly 4 groups' along with 4 control subgroups (CG1, CG2, CG3 and CG4) and 4 treated subgroups (TG1, TG2, TG3 and TG4) of each primary group, practicing different physical activities and sports were considered. Inclusion criteria included individuals aged 18 to 26 years who exercised regularly (at least four training sessions per week). Pre-existing illnesses or injuries that could hinder athletic performance or interfere with the research interventions were excluded as a criterion as shown in Figure 1.

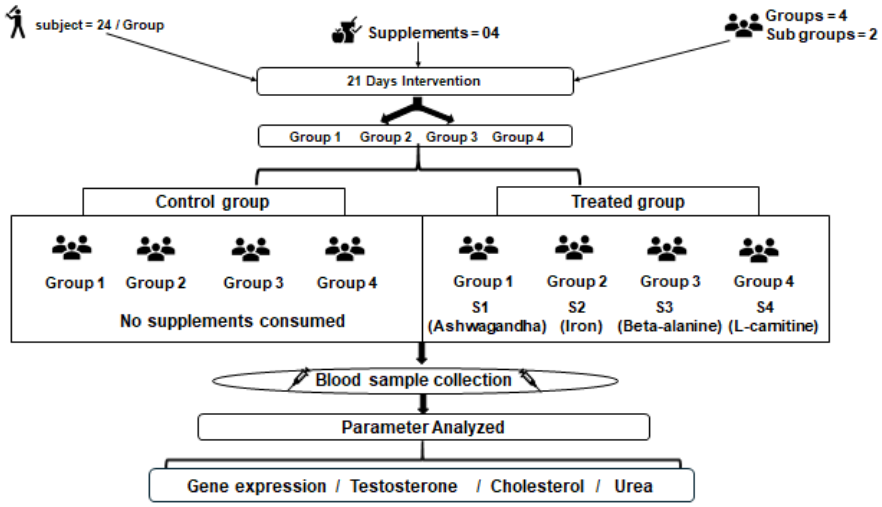

Figure 1. Diagrammatic representation, which shows the brief details of the overall process of intervention, the categories of groups, supplements consumed, and biochemical parameters analyzed

All subjects gave their voluntary written consent before participating in the study. In the present study, a 21-day dietary supplement intervention was conducted in which subjects were administered four dietary supplements, namely S1 (ashwagandha), S2 (iron), S3 (beta-alanine) and S4 (L-carnitine). All subjects were carefully monitored during the intervention period and it was ensured that they took the supplements properly within the specified time. Blood samples were taken from the subjects before and after the intervention to test the parameters, after which further verifications and testings were performed. All participants were familiar with the blood sampling techniques. All blood samples were taken during the afternoon hours as shown in Table 1.

Table 1. Groups participated in the study

Groups		Supplements taken	Intervention period
G1	CG1	NST	21 days
	TG1	S1	21 days
G2	CG2	NST	21 days
	TG2	S2	21 days
G3	CG3	NST	21 days
	TG3	S3	21 days
G4	CG4	NST	21 days
	TG4	S4	21 days

Note: *NST: No Supplements Taken; CG: Controlled Group; TG: Treated Group; S1: Ashwagandha; S2: Iron; S3- Beta-alanine; S4: L-carnitine

Quantitative real-time PCR

The TRIzol method (Ambion, Austin, TX) was used to extract total RNA from pelleted cells according to the manufacturer's instructions (Waldman et al.). The concentration of total RNA was determined using a Biotek Plate Reader (Biotek, Winooski, VT) on a Take 3 plate. Using the High-Capacity cDNA Reverse transcription Kit (Applied biosystems, Foster city, CA) and the manufacturer's instructions, cDNA was synthesized from 1 mg total RNA and 18s. The PCR mRNA calculation was then set to 18s as a control. The 7500 Fast Real-Time PCR system (Applied biosystems) was used for real-time quantitative PCR analysis. Based on the threshold transition point (Ct value), the relative expression approach was used to calculate the analysis of gene expression values, as explained in Table 2 [42]. Testosterone was measured by the kit method (calbiotech, Lot: TES6670) using ELISA.

Table 2. Primer sequence for q-PCR

Primer label	Primer sequence (Forward)	Primer sequence (Reverse)	Application
Beta-actin (Reference gene)	taagcacaccgtc-tacagca	gcaagctcggaagt-catcag	Housekeep-ing gene
Actn3	actcttccag-ccttccttcc	tctccttctgcatcct-gtcg	Sports gene

Testosterone: Testosterone was measured by the kit method (Cal biotech, Lot: TES6670) using ELISA.

Cholesterol: Cholesterol was measured using the CHOD-PAP method (with LCF), and End Point Kit (ERBA Mannheim, Lot. No: B112234) with a semi-automated biochemical analyzer.

Bun: Bun was measured by using the GLDH- urease method, initial rate kit (ERBA Mannheim, Lot. No: B092234) with a semi-automatic biochemistry analyzer.

Statistical analysis

The experiments were analyzed using one-way Analyses of Variance (ANOVA) with Bonferroni tests for multiple comparisons, and the treatment groups were compared with the control group. The data are shown as mean-SE. A statistical significance threshold of $p<0.05$ was applied.

RESULTS

Gene expression

The final values of gene expression can be found in (Table 3). In addition, the concentrations of the control and treated groups are shown in Figure 2, which shows an increase in gene expression in the treated group. Also, the mean values presented in Figure 2 shows that the supplements consumed by the treated group increased *Actn3* gene expression compared to the gene expression of the control group. The gene expression values of the all supplements are shown in Figure 2. All supplements significantly increase *Actn3* gene expression.

Table 3. Gene folds expression values of CG and TG with Standard error

Groups		Supplements taken	Gene fold expression ± SE	p-value
G1	CG1	NST	2.51913299367 ± 0.11	0.00252207*
	TG1	S1	3.37662024783 ± 0.12	

G2	CG2 TG2	NST S2	2.42962992167 ± 0.06 3.15529512800 ± 0.11	0.0000896999*
G3	CG3 TG3	NST S3	2.36530041700 ± 0.08 3.23302520717 ± 0.06	0.00028339*
G4	CG4 TG4	NST S4	2.56486425683 ± 0.07 3.21186001117 ± 0.11	0.001781613*

Note: NST: No Supplements Taken; CG: Controlled Group; TG: Treated Group; G1: Group 1; G2: Group 2; G3: Group 3; G4 : Group 4; S1: Ashwagandha; S2: Iron; S3: Beta-alanine; S4: L-carnitine; *: Significant value; SE: Standard Error

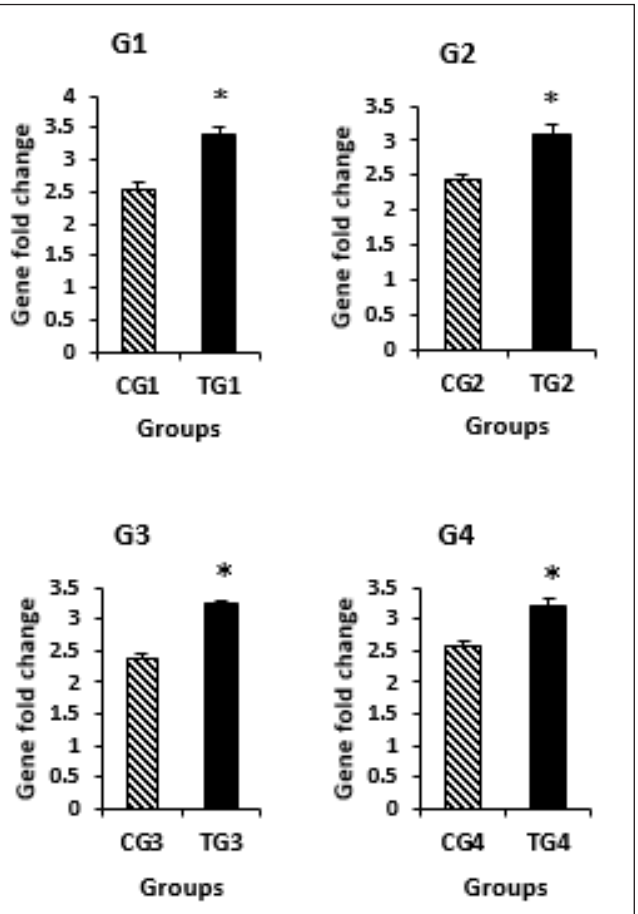

Figure 2. Gene fold expression of groups G1, G2, G3 and G4, who consumed S1, S2, S3 and S4 supplements

Total testosterone: The results has shown in the descriptive Figures 3 and 4. The final total testosterone concentrations in all the groups increased after the consumption of given supplements, but only group G3 and G4 showed significant difference. The concentrations before and after treatment are shown in the Table 4. The p-values in the Table 4 also shows that group G3 and G4 have a significant difference between the concentrations of before and after treatment, whereas, the total testosterone values of group G1 and G2 didn't showed significant result but still increased the total testosterone levels in athletes. Figure 4 shows the mean concentration values of all the supplements. The information in the Figure 4 shows that supplements taken by groups G3 and G4 increased their testosterone levels, while G1 and G2 showed no significant difference.

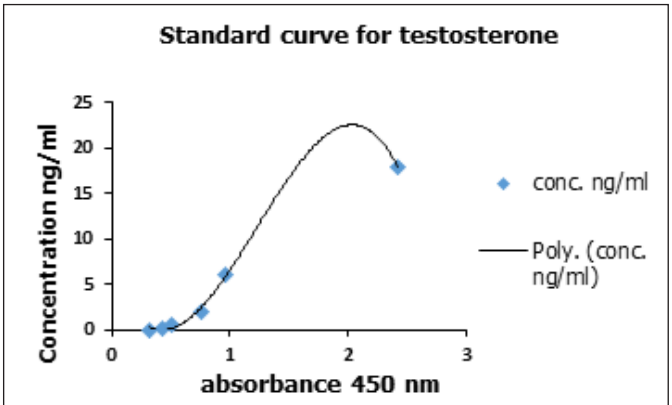

Figure 3. Standard curve values of Testosterone
Note: (◆): Conc.ng/ml ; (——): Poly.(conc. Ng/ml)

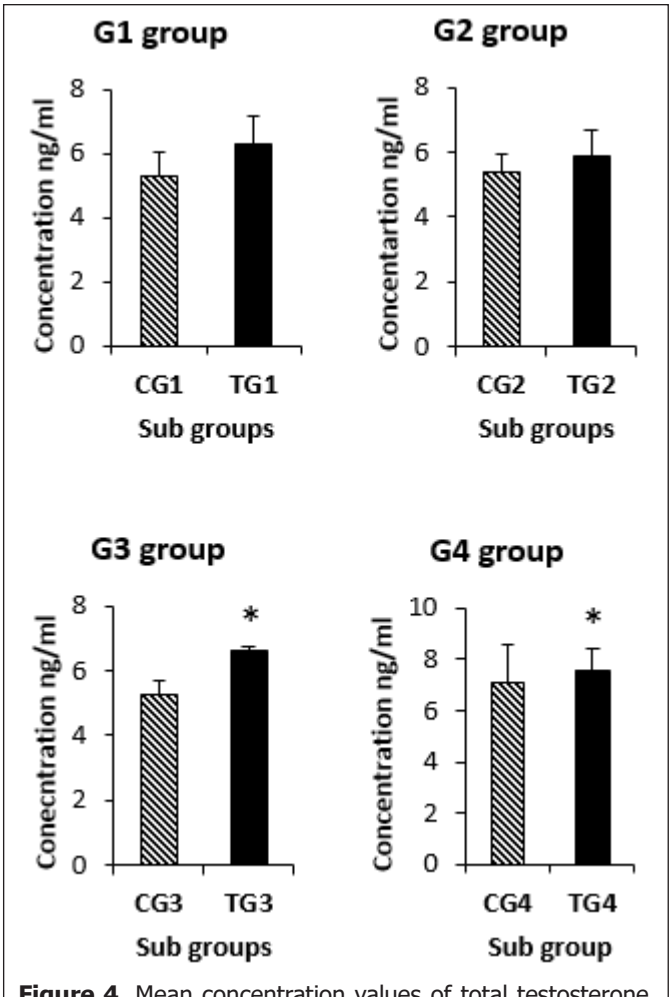

Figure 4. Mean concentration values of total testosterone of groups G1, G2, G3 and G4

Table 4. T.T values of CG and TG with Standard error

Groups		Supplements taken	T.T ± SE	p-value
G1	CG1 TG1	NST S1	5.2715 ± 0.79 6.2658 ± 0.90	0.430214
G2	CG2 TG2	NST S2	5.3666 ± 0.59 5.8546 ± 0.85	0.651701
G3	CG3 TG3	NST S3	5.2572 ± 0.42 6.608 ± 0.13	0.022875*
G4	CG4 TG4	NST S4	7.0574 ± 1.49 7.5574 ± 0.89	0.011489*

Note: *NST: No Supplements Taken; CG: Controlled Group; TG: Treated Group; G1: Group 1; G2: Group 2; G3: Group 3; G4: Group 4; S1: Ashwagandha; S2: Iron; S3: Beta-alanine; S4: L-carnitine; *Significant value: SE: Standard Error

Cholesterol: The final concentrations are listed in the Table 5. Figure 5 shows that the cholesterol levels have decreased, particularly in all the group, but only group G1 showed a significant difference. The mean concentrations of the control and treated groups are also shown in the Table 5, in which the values of all supplements, i.e. S1, S2, S3, and S4, are listed separately. All supplements contribute equally to lowering or controlling the cholesterol levels in subjects. Figure 5 shows that the concentration values in all the treated group that took the supplements decreased compared to the control group.

Table 5. Cholesterol values of CG and TG with Standard error

Groups		Supplements taken	Cholesterol ± SE	p-value
G1	CG1 TG1	NST S1	88.285 ± 5.18 69.57 ± 5.11	0.042296*
G2	CG2 TG2	NST S2	96.6475 ± 9.91 74.4025 ± 15.6	0.275536
G3	CG3 TG3	NST S3	130.91 ± 24.2 115.65 ± 25.3	0.686123
G4	CG4 TG4	NST S4	126.582 ± 6.68 99.43 ± 13.1	0.102174

Note: *NST: No Supplements Taken; CG: Controlled Group; TG: Treated Group; G1: Group 1; G2: Group 2, G3: Group 3; G4: Group 4; S1: Ashwagandha; S2: Iron, S3: Beta-alanine; S4: L-carnitine; *Significant value

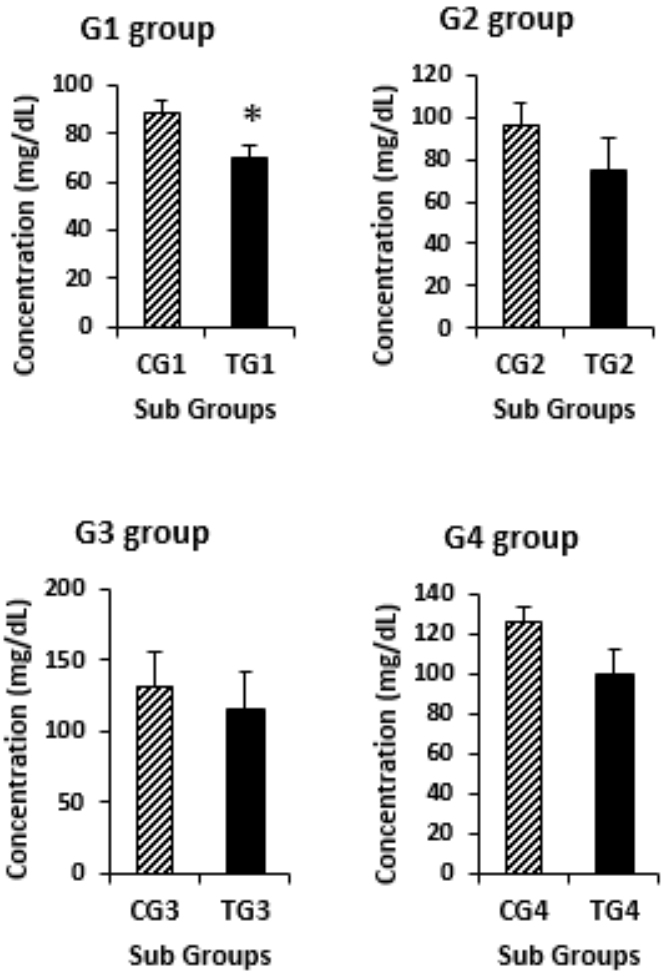

Figure 5. Mean concentration values of cholesterol of groups G1, G2, G3 and G4

Bun: The Bun urea concentrations of the samples are shown in the Table 6. The Bun concentrations of all samples are in the normal range, i.e. 19 mg/dl-45 mg/dl. The descriptive Figure 6 shows that Bun content is balanced in both the control group and the treated group. As can be seen from the Figure 6, the mean concentrations of Bun are approximately same in all the groups. Furthermore, Figure 6 also shows the concentrations of all the supplements, which shows that they equally balances the Bun content in the body. Therefore, it helps in the prevention of severe kidney failure.

Table 6. Mean urea (BUN) level of CG and TG with the Standard error

Groups		Supplements taken	BUN ± SE	p-value
G1	CG1 TG1	NST S1	31.78333333 ± 4.7 34.44166667 ± 2.8	0.686123
G2	CG2 TG2	NST S2	33.05333333 ± 7.6 31.61333333 ± 3.1	0.870003
G3	CG3 TG3	NST S3	34.05 ± 7.0 34.31 ± 4.6	0.976974
G4	CG4 TG4	NST S4	37.685 ± 9.2 32.715 ± 3.8	0.670175

Note: No Supplements Taken; CG: Controlled Group; TG: Treated Group; G1: Group 1; G2: Group 2; G3: Group 3; G4 : Group 4; S1: Ashwagandha; S2: Iron; S3: Beta-alanine; S4: L-carnitine; SE: Standard Error

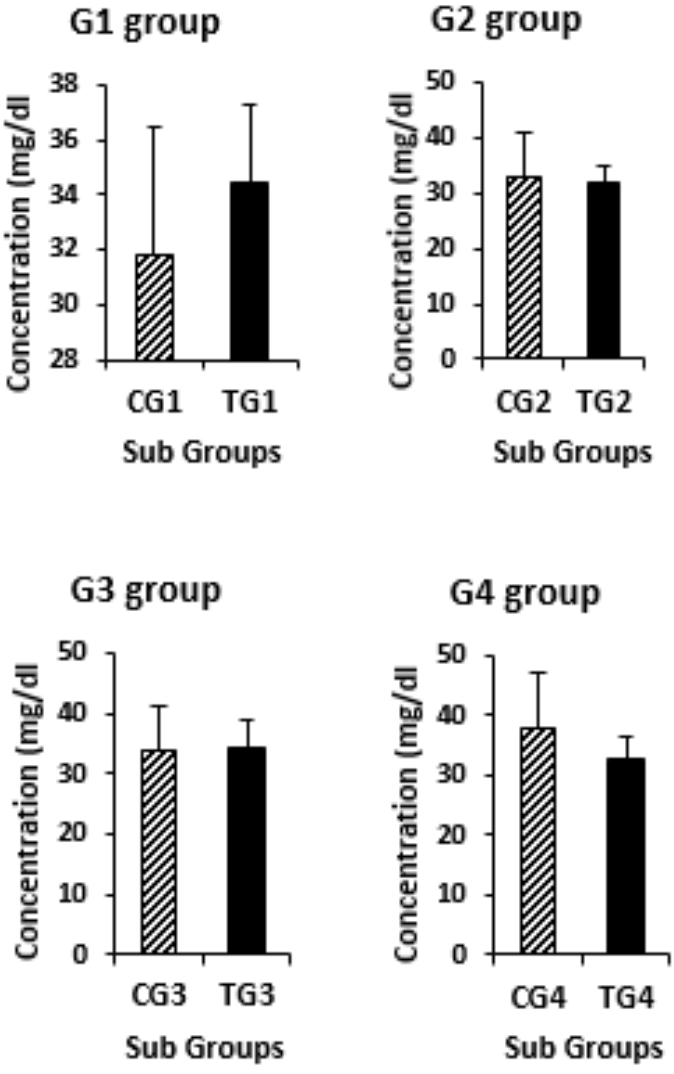

Figure 6. Mean concentration values of BUN of groups G1, G2, G3 and G4

DISCUSSION

The results show that supplements S1, S2, S3, and S4 are strong regulators of *ACTN3* gene expression. The observed changes in gene expression underscore the potential regulatory role of these nutrients in regulating genes related to muscle, particularly the *ACTN3* gene, which is associated with fast-twitch muscle fibers and force production. These results are consistent with previous studies that have found a link between dietary intake and gene expression in muscle tissue. The observed significance of the data supports the hypothesis that these nutrients may influence gene expression in a way that improves muscle function and athletic performance.

The results show that dietary supplements have a significant effect on *ACTN3* gene expression, with the G2 group having a p-value of 0.0000896999, the G1 group of 0.00252207, the G3 group of 0.00028339 and the G4 group of 0.001781613. Therefore, the null hypothesis stating that there is a significant effect of dietary supplements on *ACTN3* gene expression is accepted. Several pathways may be involved in the mechanisms underlying the observed changes in *ACTN3* gene expression.

The results also shows the potential effect of S3 and S4 on testosterone levels, while all the given supplements increases the total testosterone levels in athletes. On the other hand, all supplements lowered cholesterol levels in all groups, with only S1 showing a significant result in cholesterol levels in the treated group. All supplements administered also maintained the subjects blood urea nitrogen levels. It is well known that iron, a necessary micronutrient, contributes to energy synthesis and oxygen transport, both of which are important for muscle function. The traditional herb ashwagandha, known for its adaptive benefits, is associated with increased stress tolerance and endurance. Carnosine, a dipeptide that buffers pH changes in muscle cells during intense exercise, is a precursor to beta-alanine. L-carnitine, which is involved in the movement of fatty acids and energy metabolism, can improve muscle performance. These nutrients may have an impact on the signaling systems that control gene regulation and muscle metabolism. They could affect the co-regulatory molecules, epigenetic changes, and transcription factors that control *ACTN3* gene expression and various biochemical indicators.

CONCLUSION

These findings have implications for exercise physiology, nutrition, and athletic performance. The observed changes in *ACTN3* gene expression, hormones and biochemical indicators, suggest that these nutrients i.e., iron, ashwagandha, beta-alanine, and L-carnitine may enhance the ability of these foods to influence muscle-related molecular processes to improve muscle health, athletic ability, and function. Based on these findings, athletes, coaches, and individuals seeking to maximize muscle performance and recovery may benefit from specific nutritional approaches. The parameters have shown a statistically significant result. It shows that there is a significant difference in *Actn3* gene expression in both the control and treated groups. It also shows the difference between the testosterone levels of the control and treated groups. Ultimately, it can be concluded that the supplements taken by the treated groups increases *Actn3* gene expression and testosterone levels, decreases cholesterol levels, and balance the participants' BUN levels. In addition, this study contributes to the growing understanding of the intricate interactions between nutrients and gene expression along with various biochemical indicators. By shedding light on the specific interactions between nutrients and genes, we deepen the broader understanding of how nutrition influences the molecular processes underlying muscle physiology and performance.

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CONFLICT OF INTEREST

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