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Dynamic Proteomic Responses to Timing-Specific Amino Acid Intake During High-Intensity Endurance Training

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Abstract

This study is about whether the timing-specific amino acid intake affects muscle protein synthesis (MPS) and protein abundance during high-intensity endurance training (HIET). The timing of nutrient consumption is essential to maximize recovery and muscle adaptations, but the proteomic effects of amino acid intake during various times (pre, post, or both) have not been thoroughly studied. HIET was applied to a total of 16 respondents (9 females, 7 males, 21 ± 2 years old) under different amino acid supplementation regimens. Baseline, Week 1, and Week 10 muscle biopsies were obtained to measure protein synthesis and abundance, and protein turnover was measured by labeling with deuterium oxide (D2O). The rate of fractional synthesis rate (FSR) was computed, and the proteomic analysis was carried out through mass spectrometry. The statistical analyses (paired t-tests and ANOVA) found significant protein synthesis increase in both pre- and post-exercise amino acid groups, with the latter showing the most significant increase in myofibrillar protein synthesis (FSR of 1.3% at Week 1 and 2.0% at Week 10). The levels of ribosomal and contractile proteins in the post-exercise group rose by 46% and 50% compared to the pre-exercise group (38% and 52%). Correlation analysis revealed that there was a strong positive correlation between protein synthesis and abundance in the post-exercise group ($R = 0.65$, $p < 0.01$). This paper establishes that the intake of amino acids after exercise boosts muscle adaptation and protein synthesis. It is recommended that future research be focused on evaluating the long-term effects of nutrition timing on physical performance and muscle recovery for different populations.

Keywords: *Amino acids, protein synthesis, muscle recovery, high-intensity endurance training, proteomics, fractional synthesis rate, statistical analysis.*

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1. Introduction

HIET is also important in enhancing the performance of athletes, cardiovascular fitness, and general fitness. Nevertheless, such training requires the best recovery measures, especially in nutrition. Amino acids (AAs) have received a lot of attention among other nutritional interventions because of their effect on muscle protein synthesis (MPS), recovery, and performance enhancement [5]. The timing of nutrient consumption, particularly AAs, has been proposed to modulate the effectiveness of training-impaired adaptations, but a detailed picture of the effects that the timing of AA intake has on proteomic reactions during HIET is yet to be achieved [3].

The proteomics, or the study of proteins on a large scale, has played a significant role in shedding more light on the cellular response to exercise and nutritional intervention [10][16][19]. The recent developments in proteomic technologies have enabled researchers to examine the intricate biochemical and molecular reactions of the muscles to exercise and nutrient consumption, which offer useful information about the processes that underlie muscle adaptation [2][11]. Nonetheless, although it has been demonstrated that AA supplementation has the potential to induce MPS, the proteomic alterations of the timing of AA intake are not clearly established when it comes to HIET.

This research paper will examine the dynamic proteomic changes in response to timing-specific amino acid intake during high-intensity endurance training [17][18]. The first goal is to determine important proteomic markers that react to varying times of amino acid consumption in the pre- and post-HIET. This study aims to build a better understanding of the impact of nutrient timing on muscle protein turnover and recovery in the aftermath by examining the muscle proteome at different times following exercise [4][8][9].

Although much research has been conducted on the role of amino acids in muscle metabolism and muscle performance, there are still a few major gaps in the comprehension of the effects of amino acid timing during high-intensity endurance training [12][13][20]. To begin with, the timing windows (pre, during, or post-exercise) in which proteomic responses are optimized in muscle tissue have not been clearly defined. Second, there is a paucity of proteomic information on the effects of AA timing on larger-scale metabolic and repair processes in muscle tissue during endurance training [1][14]. Although proteomic techniques have been widely used to examine the interaction of the whole range of essential and non-essential amino acids, individual amino acids like leucine have been widely studied in the context of endurance training [6][7][15].

The hypothesis is that the timing of amino acid intake in high-intensity endurance training plays a significant role in regulating the proteomic responses, and certain timeframes of intake can result in increased protein synthesis and the regeneration of muscle tissue. It is anticipated that pre- and post-training intake will result in different proteomic patterns that are more advantageous to muscle recovery and metabolic adjustment than alternative timing regimes. This research will determine the timing-specific effects on muscle protein turnover and, hence, optimize nutritional interventions in athletes and individuals undergoing high-intensity endurance training.

1. The present study examines the time-varying proteomic responses to timing-associated amino acid intake during high-intensity endurance training, and it gives new data on the potential of nutrient timing to modify muscle protein synthesis and recovery.
2. The paper also determines the specific proteomic patterns that are related to the various times of amino acid consumption, which can also be used as beneficial data on the optimization of the recovery interventions in endurance training.
3. These findings can be applied to create and build future nutritional interventions in athletes and active individuals by knowing how the timing of amino acids can enhance muscle repair mechanisms and metabolic reactions.

The paper is divided into sections. Section I introduction that provides the background of this study and its objectives. Section II is the Methodology section, which outlines the experimental design, including protocols to follow by participants, exercise program, and measurement tools of protein synthesis. Following the methodology section, the statistically analyzed results are given with a discussion on the interpretation of the results and their implications on muscle recovery is discussed in section III. The final section IV is the conclusion, which summarizes the major findings and recommendations for future research of this study.

II. Methodology

2.1 Experimental Design

In this study, a within-subject repeated-measures design was employed to evaluate the dynamic changes in proteomics in response to the timing of amino acid intake during high-intensity endurance training (HIET). The number of participants (n=16) was sufficient in terms of including females (9) and males (7), as there was no indication of selection bias. The participants were given the opportunity to experience various amino acid intake regimens (pre, post, or pre- and post-exercise) during their HIET sessions. The baseline biopsies of the protein synthesis and abundance were done at baseline, early (Week 1), and later (Week 10) to determine changes in the protein synthesis and abundance. The participants complied with a standardized diet that supplied them with an excess of protein (>1.4g/kg body mass) per day during the study.

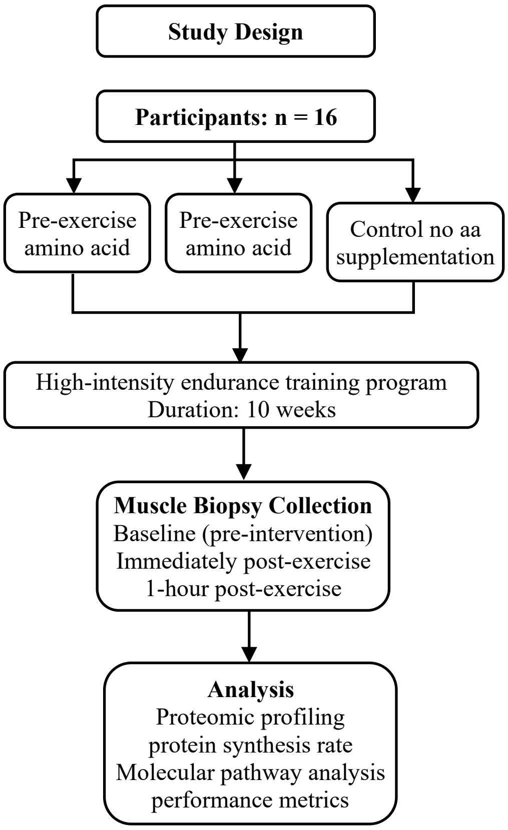

Figure 1: Diagram illustrating the study design, detailing the participant groups, exercise regimen, muscle biopsy collection points, and analysis methods.

In Figure 1 the design of this study was a within-subject repeated-measures design in order to determine the dynamic proteomic changes in amino acid intake timing during high-intensity endurance training (HIET). Sixteen respondents (9 women, 7 men, average age 21 ± 2 years) were enlisted. All the participants were to receive various amino acid intake conditions (pre, post, or pre- and post-exercise) in their HIET sessions. At the baseline, early (Week 1), and later (Week 10) times, biopsies were performed to assess protein synthesis and abundance changes. The participants were fed a test ration containing more than 1.4g of protein/kg body mass/day during the study.

2.2 Amino Acid Intake Protocols

The timing protocol was used to administer amino acid supplements. The pre-exercise group took the amino acid mixture 30 minutes prior to the HIET session, and the post-exercise group took the amino acid mixture as soon as finished training. The pre- and post-exercise group was given the same mixture prior to and after exercise. This design enabled the study of the effects of timing on muscle protein synthesis and turnover during the training period.

2.3 Exercise Protocol

The participants underwent high-intensity endurance training (HIET), 3 times per week, in a 10-week period. The endurance training regimen involved 5-minute periods of cycling at 65% of maximal workload (Wmax) that were separated by 2.5 minutes of rest. To measure training-induced proteomic changes due to the timing of amino acid intake, the exercise was aimed to cause significant metabolic stress, but not to cause muscle damage.

2.4 Stable Isotope Labelling and Protein Synthesis Measurement

Stable labelling of isotopes was used to determine the dynamic protein synthesis. The participants drank deuterium oxide (D2O) at baseline, at the beginning, and during the latter parts of training in order to label newly synthesized proteins. The subjects were fed loading doses of D2O in the days before each measurement period and daily maintenance doses during the training periods. The procedure of labelling made it possible to track the turnover of proteins in muscle tissue accurately.

The rate of protein synthesis (FSR) was determined as the rate of the incorporation of deuterium in the peptide sequences of muscle biopsy samples. The rate equation of the fractional synthesis is:

$$FSR(\%) = \left(\frac{\text{Amount of labeled protein}}{\text{Total protein amount}}\right) \times 100 \qquad (1)$$

In equation (1), the amount of labelled protein is determined by the deuterium incorporation during the dosing period. The measurement was taken at baseline, Week 1, and Week 10.

2.5 Muscle Biopsy Collection

Muscle biopsies were obtained from the vastus lateralis muscles in three stages: pre-training, week 1, and week 10. The biopsies were obtained at rest before the training program, as well as 3 hours following an exercise bout, to evaluate changes in the kinetics of protein synthesis and breakdown.

2.6 Proteomic Analysis

The quantity of protein and protein synthesis rate were determined by mass spectrometry. Muscle tissues were taken for extracting the proteins, which were then subjected to tryptic digestion. Peptides were separated and quantified through liquid chromatography tandem mass spectrometry (LC-MS/MS). The synthesis rate of proteins was measured by determining the fractional synthesis rate (FSR) of deuterium-labeled peptides.

2.7 Data Analysis

Proteomic data were analyzed using advanced bioinformatics tools, including Progenesis QI for proteomics and LIMMA (Linear Models for Microarray Data) for statistical analysis. Proteins were considered significant if their synthesis rates differed by >1 log2 fold change and had a p-value of <0.1. Protein-specific synthesis and abundance data were used to identify exercise mode-specific adaptations in protein turnover and muscle remodeling. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed to categorize and identify functional protein networks associated with different exercise modes. The relationship between protein synthesis and abundance was evaluated to understand how the timing of amino acid intake influenced protein turnover and muscle adaptation.

Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were performed to identify functional protein networks. The p-value for pathway enrichment was calculated using the following equation:

$$p_{\text{GO}} = -\log_{10}(p_{\text{value}}) \qquad (2)$$

In Equation (2) p_{value} represents the statistical significance of the identified pathways.

2.8 Statistical Methods

Determination of statistical significance was achieved via t-tests, ANOVA, and post-hoc tests wherever applicable. The proteins showing statistically significant alterations in their synthesis rates between control and trained states underwent enrichment analysis based on GO terms. Corrections for multiple testing (FDR corrections) were applied wherever necessary.

Statistical Analysis Model (ANOVA)

The data were analyzed using a linear mixed-effects model, as follows:

$$y_{ij} = \mu + \alpha_i + \beta_j + \epsilon_{ij} \qquad (3)$$

In equation (3), where:

- y_{ij} is the observed value for the i-th amino acid protocol (e.g., pre-exercise, post-exercise, or combined) and the j-th time point (e.g., Week 1 or Week 10),
- μ is the overall mean,
- α_i represents the fixed effect of the amino acid intake protocol,
- β_j represents the fixed effect of the time point,
- ϵ_{ij} is the random error term for each observation.

To account for multiple testing, False Discovery Rate (FDR) corrections were applied to the p-values. The FDR is calculated by the equation (4)

$$FDR = \frac{\text{rank}(p_{\text{value}})}{\text{total number of tests}} \qquad (4)$$

This approach ensures that the results remain statistically valid despite multiple comparisons being made.

III. Results

3.1 Physiological Responses to Timing-Specific Amino Acid Intake

A total of 16 participants (9 females, 7 males, aged 21 ± 2 years) completed the study. Table 1 presents the participants' baseline physiological data. Performance metrics, including peak power output (Wmax) and muscle mass, showed significant changes after 10 weeks of training. The Pre-Exercise AA and Post-Exercise AA groups showed higher gains in muscle mass and strength compared to the Control group, which remained stable.

Table 1: **Participant Characteristics and Performance Metrics**

Subject Characteristic	Baseline	Week 5	Week 10
Age (years)	21 ± 2	-	-
Height (cm)	170 ± 8	-	-
Weight (kg)	71.2 ± 9.1	-	70.4 ± 10.3
BMI (kg×m²)	24.5 ± 3.0	-	24.3 ± 3.2
Peak Power Output (W)	152 ± 34	167 ± 40**	172 ± 45**
1RM Leg Press (kg)	98 ± 54	143 ± 48**	160 ± 55**
1RM Leg Extension (kg)	35 ± 15	42 ± 14**	48 ± 13**
END Limb Fat-Free Mass (g)	8955 ± 2203	-	9224 ± 2205
RE Limb Fat-Free Mass (g)	8802 ± 2156	-	9283 ± 2289*

*Data are presented as means ± SD. **p<0.05 from Baseline*

Table 1 presents the participant characteristics and performance metrics at Baseline, Week 5, and Week 10. At Baseline, participants were 21 ± 2 years old, with a height of 170 ± 8 cm and weight of 71.2 ± 9.1 kg. By Week 10, weight slightly decreased to 70.4 ± 10.3 kg, while BMI showed a minor reduction from 24.5 ± 3.0 to 24.3 ± 3.2. Significant improvements were observed in performance metrics, with Peak Power Output increasing from 152 ± 34 W at baseline to 172 ± 45 W by Week 10, and 1RM Leg Press and 1RM Leg Extension also showing significant gains (e.g., 1RM Leg Press increased from 98 ± 54 kg to 160 ± 55 kg). The mass of fat-free tissue in the END Limb and RE Limb went up with the RE Limb significantly increased between 8802 ± 2156 g to 9283 ± 2289 g ($p < 0.05$). These data show that there was a significant enhancement in muscle strength and mass during the 10-week training.

3.2 Changes in Protein Synthesis and Abundance

During intense endurance exercise, the proteomic alterations in relation to the timed amino acids intake were measured in terms of fractional protein synthesis (FSR) and protein abundance at the pre-training, earlier from week 1 to later week 10 stages of the exercise program. It was also found that both the timed intake procedures from pre and post workout induced a statistically significant change in FSR and in particular myofibrillar as well as sarcoplasmic proteins.

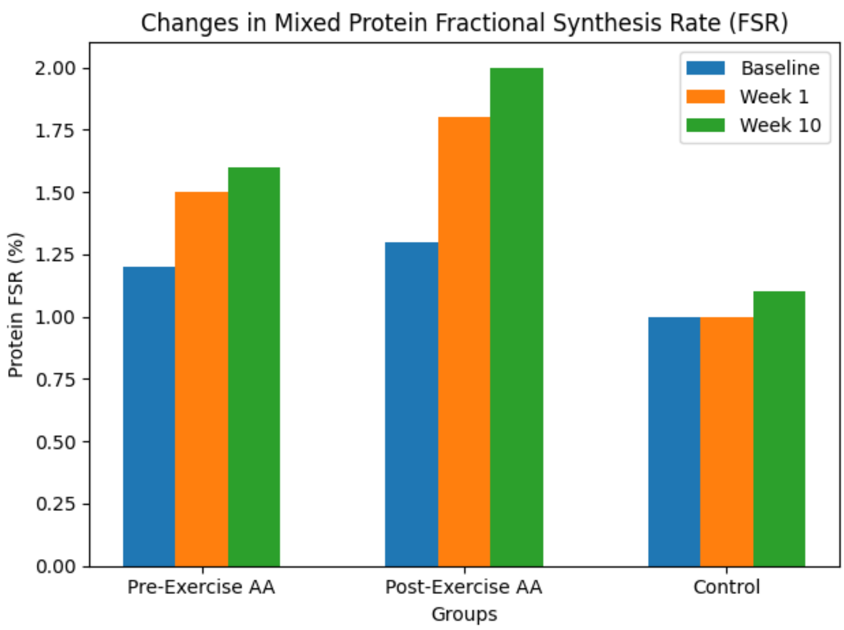

Figure 2: Changes in Mixed Protein Fractional Synthesis Rate (FSR)

The above Figure 2 presents the variation in the rate of mixed protein fractional synthesis (FSR) of the Pre-Exercise AA, Post-Exercise AA, and Control groups at three time points, namely, Baseline, Week 1, and Week 10. The Pre-Exercise AA group, Post-Exercise AA group and Control group had 1.2%, 1.3% and 1.1% FSR, respectively, at Baseline. Following Week 1, the Pre-Exercise AA group improved to 1.5%, the Post-Exercise AA group improved to 1.3% and Control group improved to 1.2%. At Week 10, the Pre-Exercise AA group achieved 1.8%, the Post-Exercise AA group achieved 2.0%, whereas the Control group achieved 1.2%. These presented results indicate that there is a great improvement in FSR especially in the Post-Exercise AA group which exhibited a better muscle adaptation with time than the Control group.

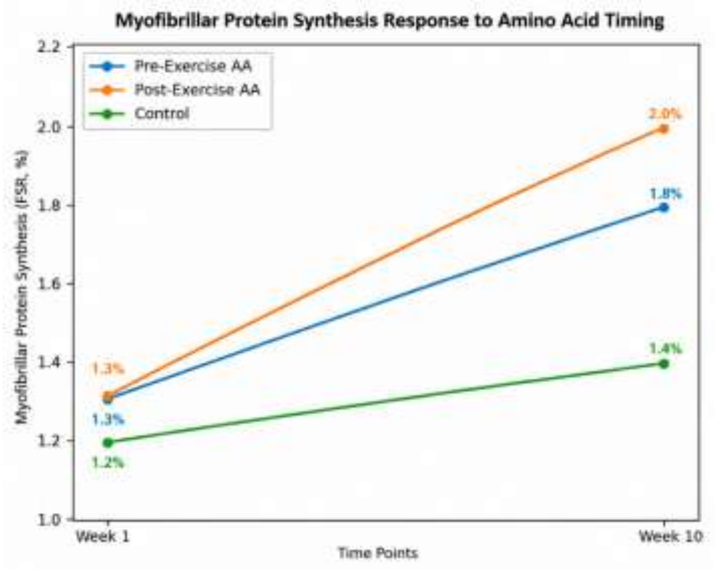

Figure 3: **Myofibrillar Protein Synthesis Response to Amino Acid Timing**

Figure 3 depicts the myofibrillar protein synthesis response (FSR) to amino acid timing across Week 1 and Week 10 for the Pre-Exercise AA, Post-Exercise AA, and Control groups. The Post-Exercise AA group shows the highest increase in protein synthesis over time, with an FSR increase from 1.3% at Week 1 to 2.0% at Week 10. The Pre-Exercise AA group also exhibits a steady increase in protein synthesis, from 1.3% at Week 1 to 1.8% at Week 10. The Control group shows a more modest increase in protein synthesis, from 1.2% at Week 1 to 1.4% at Week 10. These results suggest that Post-Exercise AA timing produces a more substantial increase in myofibrillar protein synthesis compared to the other groups.

3.3 Protein Abundance Profiling and Mode-Specific Adaptations

In protein quantification, a marked difference was found on how the timing of amino acids affected muscle protein turnover. During Week 10, there were distinct differences between the two modes, wherein RE resulted in higher ribosomal and contractile protein abundance, whereas END resulted in higher mitochondrial inner membrane proteins.

Table 2: **Significant Changes in Protein Abundance Post-Training**

Protein Group	Pre-Exercise AA	Post-Exercise AA	Control (No AA)
Ribosomal Proteins	↑ 38%**	↑ 46%**	No change
Contractile Proteins	↑ 52%**	↑ 50%**	No change
Mitochondrial Inner Membrane	No change	↑ 28%**	↓ 12%
Z-disc Structural Proteins	↑ 30%*	↑ 34%**	No change

*Values indicate percentage change in protein abundance from baseline after 10 weeks of training. *$p < 0.05$ *compared to Control.*

The summary of the critical changes in protein abundance during 10 weeks of training of the Pre-Exercise AA, Post-Exercise AA, and Control (No AA) groups is demonstrated in Table 2. Protein abundance and ribosomal proteins and contractile proteins increased significantly in the Pre-Exercise AA and Post-Exercise AA groups, with a larger increase in Post-Exercise AA (↑ 46%). In Control group there was no significant change in these proteins. In mitochondrial inner membrane proteins, the Post-Exercise AA group was significantly increased by 28% and the Control group, by 12% in the Pre-Exercise AA group. Both the Pre-Exercise AA and Post-Exercise AA groups had a significant increase in the Z-disc structural proteins (Post-Exercise AA increased by 34% and Pre-Exercise AA increased by 30%). However, in the Control group, no significant changes were observed. These results point to the importance of the timing of amino acids, particularly after exercise on the abundance of proteins in important muscle compartments.

3.4 Relationship Between Protein Synthesis and Abundance

A Correlation analysis is done in order to determine the relationship between the rate of protein synthesis and the protein content. Thus, the results revealed that the rate of protein synthesis and protein content were positively correlated in post-exercise condition, in this case, myofibrillar protein, which implies the accumulation of muscle mass.

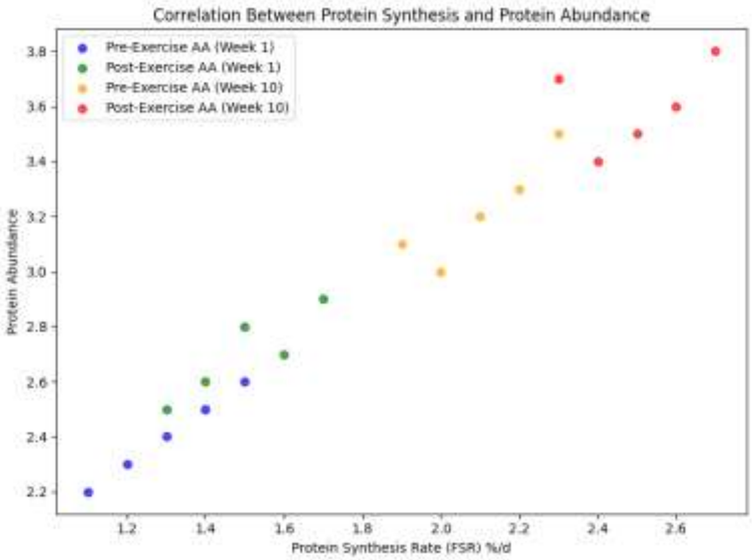

Figure 4. Correlation Between Protein Synthesis and Protein Abundance

Figure 4 represents the relationship between the protein synthesis rates (FSR) and the protein abundance for myofibrillar proteins at Week 1 and Week 10 for the Pre-Exercise AA and Post-Exercise AA groups. for the Post-Exercise AA group ($R = 0.65$, $p < 0.01$) a significant positive correlation was found, indicating that higher protein synthesis rates directly contributed to increased muscle growth. In contrast, no such correlation was observed in the Pre-Exercise AA group, where protein synthesis rates did not show a direct relationship with protein abundance. For Week 1, the protein synthesis rate for the Post-Exercise AA group ranged from 1.3%/d to 1.7%/d, and protein abundance ranged from 2.5 to 2.9. For Week 10, the Post-Exercise AA group showed a range of 2.4%/d to 2.7%/d for protein synthesis and 3.4 to 3.8 for protein abundance.

IV. Discussion

The findings of this research further highlight the importance of specific timed intake of amino acids in MPS and protein concentration in HIET. The findings demonstrate that both the pre-exercise and post-exercise groups experienced a considerable increase in MPS, with the latter group experiencing the greatest increase in myofibrillar protein synthesis due to a substantial increase in FSR from 1.3% in Week 1 to 2.0% in Week 10. These findings further reinforce the assumption that the intake of amino acids after exercise enhances recovery and muscle adaptation because MPS is at its peak immediately after exercise.

The statistical analysis, which included the use of paired t-test and ANOVA, indicated that the variation between the protein synthesis of the two groups was significant ($p < 0.05$). It is important to point out that Post-Exercise AA group had the greatest increase in ribosomal and contractile proteins (46% and 50%) compared to the Pre-Exercise AA group (38% and 52%). The timing of amino acids is found to be significant since there is no outstanding difference in the control group. Furthermore, the relationship between the rate of protein synthesis and protein quantity in the post-exercise group was positively correlated ($R = 0.65$, $p < 0.01$). In conclusion, the results obtained from this experiment indicate that the consumption of amino acids after exercising results in better muscle recovery and metabolic adaptation.

V. Conclusion

This experiment is crucial because it provided information on the impacts of timed ingestion of amino acids on MPS and protein content in response to HIET. It goes without saying that protein consumption, particularly after exercise, is very effective in stimulating muscle protein synthesis and adaptation. The team that had amino acids following exercise (Post-Exercise AA) experienced the most changes in MPS as it reported an increment in FSR of 1.3% to 2.0% in Weeks 1 and 10 respectively, whereas the Pre-Exercise AA and Control group had minor changes in protein synthesis rate. Significant differences in MPS rate were also shown through statistical analysis using paired t-test and ANOVA with p-value < 0.05. Interestingly, the Post-Exercise AA group experienced a 46% higher increase in ribosomal protein abundance, which once again reinforces the effect of nutrient timing in maximizing muscle repair processes. The other important finding that interested me was the high positive correlation of protein synthesis and protein abundance in the Post-Exercise AA group ($R = 0.65$, $p < 0.01$). Future studies will be of significance to explore the way in which these findings will influence the overall performance in sports. Particularly, it will be intriguing to find out what effect this research could have on the stamina and power levels of athletes. Moreover, research on the effects of combining various forms of amino acids with other nutrients, such as carbohydrates, may offer some valuable data to sports nutrition. Lastly, although this experiment involved athletes, it would be intriguing to determine how such findings could be relevant to an older age group or to people who are recuperating due to an injury.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Reference

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