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Alterations in Blood Levels of Vitamin B2, B12, and Folate Following Acute Exercise-Induced Elevation of Plasma Homocysteine

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Abstract

Acute exercise is known to influence several biochemical parameters, including plasma homocysteine (Hcy), a sulfur-containing amino acid linked to cardiovascular risk. The metabolism of Hcy is dependent on several B-vitamins, notably vitamin B2 (riboflavin), B12 (cobalamin), and folate (vitamin B9), which act as coenzymes or methyl donors in one-carbon metabolic pathways. This study examined how a single session of moderate-to-intense aerobic exercise alters the levels of these vitamins and whether these changes are associated with shifts in plasma homocysteine. Twenty healthy young adults performed a 45-minute treadmill exercise at 75% of maximum heart rate. Blood samples were collected at baseline, immediately post-exercise, and one hour post-exercise. The findings revealed a significant increase in homocysteine levels immediately post-exercise, accompanied by reductions in serum vitamin B2 and folate. No significant change was observed in vitamin B12. These findings suggest that acute exercise induces short-term disruptions in B-vitamin status that may contribute to transient elevations in homocysteine, underlining the need for monitoring nutritional status in physically active individuals.

1. Introduction

Regular physical activity is widely recognized as a cornerstone of good health. However, acute exercise can induce temporary physiological stress, including changes in oxidative status, inflammation, and metabolic biomarkers. One such biomarker is **homocysteine (Hcy)**, a non-proteinogenic amino acid formed during methionine metabolism. Elevated plasma homocysteine, even transiently, has been associated with endothelial dysfunction, increased oxidative stress, and elevated cardiovascular risk [3].

Homocysteine metabolism is primarily regulated through two pathways: **remethylation** and **transsulfuration**. These pathways depend heavily on the availability of specific B-vitamins. Folate and vitamin B12 are essential for the remethylation of Hcy to methionine, while vitamin

B2 serves as a coenzyme for methylenetetrahydrofolate reductase (MTHFR), which is required for folate cycling [2]. Deficiencies or functional impairments in any of these vitamins can result in impaired homocysteine metabolism and increased circulating levels.

The effects of acute exercise on homocysteine levels have been observed in both sedentary and athletic populations. However, less is known about how B-vitamin concentrations respond concurrently, and whether these changes influence post-exercise homocysteine dynamics. Understanding these responses is essential for optimizing recovery and long-term cardiovascular health in active individuals.

This study investigates the impact of acute aerobic exercise on plasma homocysteine and the circulating levels of vitamin B2, B12, and folate in healthy adults.

2. Materials and Methods

2.1 Participants

Twenty healthy, non-smoking adults (10 men, 10 women), aged 20–35 years, participated in the study. All subjects engaged in moderate exercise (3–5 sessions per week) but were not elite athletes. None had taken vitamin supplements within the past three months.

Table 1. Participant Characteristics

Characteristic	Mean ± SD
Age (years)	26.4 ± 3.8
BMI (kg/m²)	22.8 ± 2.3
VO_2 max (ml/kg/min)	45.1 ± 6.4
Sex (M/F)	10 / 10
Training frequency	4.2 ± 1.1 days/wk

2.2 Study Design

The study followed a repeated-measures, within-subject design. Participants arrived after an overnight fast and avoided caffeine and strenuous activity for 24 hours before testing. Each performed a treadmill run at 75% HRmax for 45 minutes.

Venous blood samples were taken at:

- **T0 (Baseline)**: before exercise
- **T1**: immediately post-exercise
- **T2**: one hour post-exercise

2.3 Laboratory Analysis

- **Plasma homocysteine** was analyzed using **high-performance liquid chromatography (HPLC)**.
- **Vitamin B2, B12, and folate** concentrations were measured using **ELISA kits** (Cloud-Clone Corp., USA) validated for human serum.

2.4 Statistical Analysis

Data were analyzed using repeated-measures ANOVA with Bonferroni post-hoc testing. Pearson correlation coefficients were used to examine relationships between variables. A p-value of < 0.05 was considered statistically significant.

3. Results

3.1 Homocysteine Response

Plasma homocysteine levels increased significantly immediately after exercise and remained slightly elevated at one hour post-exercise.

3.2 Vitamin Level Changes

Significant decreases were observed in serum vitamin B2 and folate immediately post-exercise. Vitamin B12 levels did not change significantly.

Table 2. Changes in Biomarkers Over Time

Biomarker	T0 (Baseline)	T1 (Post-exercise)	T2 (1h Post-exercise)	*p*-value
Homocysteine (µmol/L)	8.1 ± 1.4	**10.2 ± 1.7**	9.3 ± 1.5	< 0.001
Vitamin B2 (ng/mL)	17.4 ± 2.1	**15.6 ± 1.9**	16.8 ± 2.0	0.012
Folate (ng/mL)	11.2 ± 1.8	**9.4 ± 1.6**	10.1 ± 1.7	0.008
Vitamin B12 (pg/mL)	410 ± 75	407 ± 73	405 ± 70	0.774 (NS)

Bold indicates statistically significant change from baseline.

3.3 Correlation Analysis

- Homocysteine levels at T1 were negatively correlated with:
  - **Folate** ($r = -0.46$, $p = 0.038$)
  - **Vitamin B2** ($r = -0.43$, $p = 0.049$)
- No significant correlation with vitamin B12 ($r = -0.10$, $p = 0.65$)

4. Discussion

The present study demonstrates that acute aerobic exercise leads to a transient elevation in plasma homocysteine levels and modest decreases in circulating folate and vitamin B2. These findings are consistent with the proposed role

of B-vitamins in maintaining homocysteine homeostasis, especially under physiological stress.

4.1 Homocysteine and Exercise

The increase in plasma homocysteine post-exercise aligns with previous findings suggesting that physical exertion induces temporary methylation imbalances and oxidative stress [5]. Elevated homocysteine levels, although short-lived, may temporarily increase endothelial stress, particularly in individuals with borderline B-vitamin status.

4.2 Folate and Vitamin B2 Depletion

The decline in folate and vitamin B2 could be due to increased demand for methylation reactions or redistribution from plasma to tissues. Riboflavin (B2), as a coenzyme for MTHFR, may be rapidly utilized in regenerating 5-methyltetrahydrofolate—the active form of folate needed for remethylating homocysteine.

These findings mirror those of [1], who noted transient declines in vitamin B2 and folate following high-intensity interval training. Furthermore, [2] observed that lower folate levels were associated with exaggerated homocysteine responses in endurance athletes.

4.3 Vitamin B12 Stability

Vitamin B12 levels remained unchanged, which may reflect its longer half-life, hepatic storage, and slower turnover. This suggests that short-term exercise may not significantly affect B12 status unless there is a pre-existing deficiency.

4.4 Implications

The implications of transient B-vitamin reductions and homocysteine elevation in healthy individuals remain unclear. However, repeated exposure to these changes during regular exercise might stress one-carbon metabolism, particularly in populations with marginal B-vitamin intake or genetic polymorphisms affecting folate metabolism (e.g., MTHFR C677T variants).

Athletes and highly active individuals may benefit from dietary strategies that ensure adequate intake of B2, B12, and folate, particularly during periods of intense training.

5. Conclusion

Acute moderate-to-intense aerobic exercise transiently elevates plasma homocysteine levels and decreases circulating concentrations of vitamin B2 and folate. These changes suggest increased metabolic demand for these vitamins during exercise. Vitamin B12 levels remain stable in the short term. These findings emphasize the importance of nutritional support for active individuals to maintain optimal methylation and cardiovascular health.

9. References

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