International Journal of Sport Nutrition and Exercise Metabolism, 2005, 15, 433-441

Altered Vitamin B_{12} Status in Recreational Endurance Athletes

Markus Herrmann, Rima Obeid, Juergen Scharhag, Wilfried Kindermann, and Wolfgang Herrmann

This study aimed to compare the vitamin B_{12} and folate status of recreational endurance athletes and inactive controls by modern biomarkers. In 72 athletes (38 ± 7 y) and 46 inactive controls (38 ± 9 y) serum levels of vitamin B_{12}, methylmalonic acid (MMA), holotranscobalamin II (holoTC), folate, and homocysteine (Hcy) were measured. Vitamin B_{12} and folate levels of both groups were comparable, but athletes had higher median (25.–75. percentile) MMA [242 (196 to 324) versus 175 (141 to 266) nmol/L] and holoTC concentrations [67 (52 to 93) versus 55 (45 to 70) pmol/L] than controls. Hcy was slightly lower in athletes [9.2 (7.2 to 12.6) versus 10.8 (8.9 to 12.9) nmol/L]. In controls, we found the following correlations: vitamin B_{12} and MMA (r = –0.38), vitamin B_{12} and holoTC (r = 0.51), MMA and holoTC (r = –0.36). In athletes, MMA did not correlate with vitamin B_{12} and holoTC. Our data suggests an altered vitamin B_{12} metabolism in recreational athletes that needs further investigation.

Key Words: vitamin B_{12}, methylmalonic acid, holotranscobalamin, folate, homocysteine

Recreational as well as elite athletes with a normal Western diet frequently use vitamin supplements (19, 23). Most of these preparations contain vitamin B_{12} and folate. Since there are only a few studies investigating the vitamin B_{12} and folate status in athletes, an evidence-based recommendation for vitamin B_{12} and folate supplementation is actually not justified (8, 9, 29). A previous study by Singh et al. indicates, however, that vitamin B_{12} metabolism might be altered in endurance athletes (26).

Vitamin B_{12} and folate are important co-factors of the methionine pathway, the most important source of methyl groups in the human organism (10, 25). Many substrates centrally involved in physical exercise, such as catecholamines, creatine, and DNA, obtain their methyl groups from this pathway (3, 4, 28). All previous studies investigating vitamin B_{12} in athletes measured vitamin B_{12} directly. Compared to modern markers of vitamin B_{12} status, however, such as holotranscobalamin II (holoTC) and methylmalonic acid (MMA), the sensitivity and specificity

Herrmann, Obeid, and Herrmann are with the University Hospital of Saarland, Dept of Clinical Chemistry and Laboratory Medicine, Homburg/Saar 66421,Germany. Scharhag and Kindermann are with the Institute of Sports and Preventive Medicine, University of Saarland, Saarbruecken 66123, Germany.

of common vitamin B_{12} measurement is limited (5, 13, 14, 21). Especially for vitamin B_{12} levels < 300 pmol/L, the clinical performance of holoTC and MMA is considerably better than direct measurement of vitamin B_{12} (14). The limitations of common vitamin B_{12} detection are mainly due to the fact that only 6 to 20% of circulating vitamin B_{12} is metabolically active (12). Moreover, it has been shown that the extracellular vitamin B_{12} level is not always correlated with the intracellular vitamin B_{12} supply (13, 14).

Hcy is the final product of the methionine pathway and can be cleared by the transsulfuration or the re-methylation pathway (10, 25). The vitamin B_{12} dependent re-methylation recycles Hcy by the transfer of a methyl group from 5-methyltetrahydrofolate, which results in the formation of methionine. The transsulfuration couples Hcy with serine to form cystathionine, which is then cleaved into cysteine and α-ketobutyrate. Methylmalonic acid is a metabolite in the degradation cascade of α-ketobutyrate. The conversion of methylmalonyl-CoA into succinyl-CoA, catalyzed by the L-methylmalonyl-CoA-mutase, is an exclusively vitamin B_{12} dependent step during the degradation of α-ketobutyrate (27). It has been shown that vitamin B_{12} deficiency can result in an accumulation of MMA prior to the enzyme (2). Therefore, MMA is used as a marker of intracellular vitamin B_{12} deficiencies.

Serum vitamin B_{12} is bound to three different transport proteins, transcobalamin I, II, and III. Only the complex of vitamin B_{12} and transcobalamin II, named holotranscobalamin II (holoTC), can be utilized by all cells. Therefore, holoTC represents the metabolically active fraction of circulating vitamin B_{12} (24).

Since MMA, holoTC, and Hcy have never been used to estimate vitamin B_{12} and folate status in athletes, we studied the supply and utilization of these 2 vitamins in recreational endurance athletes and inactive controls using the above-mentioned modern biomarkers.

Materials and Methods

Subjects and Design

In the present study, we investigated serum levels of vitamin B_{12}, MMA, holoTC, Hcy, and folate in 72 healthy recreational endurance athletes (64 men and 8 women) and 46 nonexercising healthy controls (34 men and 12 women), who did not use vitamin supplements. The study design was approved by the institutional review board. All subjects gave written informed consent.

Blood Samples and Assays

Nonfasting venous blood samples were taken at rest. In athletes, blood sampling was done at least 8 h after the last training. After 20 min of coagulation, serum was immediately separated from blood cells by centrifugation and transported on ice to our laboratory, where samples were stored at –20 °C until analysis.

Vitamin B_{12} and folate were measured with commercial, competitive chemiluminescence immunoassays (Bayer Diagnostics, Fernwald, Germany) on an ACS Centaur automated analyzer (Bayer Diagnostics). The folate assay detects 5-methyltetrahydrofolate. Inter- and intraassay CVs were < 4.0 and < 4.4% for vitamin B_{12} and < 5.5 and < 5.3% for folic acid, respectively. Hcy was detected by a commercial HPLC assay (Immundiagnostik, Bensheim, Germany) with fluorescence detection.

MMA was assayed by a modified capillary gas chromatography-mass spectrometry method (capillary gas chromatograph, model 6890 with a Hewlett-Packard model 5973 mass-selective detector) according to the method described by Allen et al. (1). Inter- and intraassay CVs were ≤ 7.2% for Hcy and ≤ 3.3% for MMA. HoloTC measurement was done with a commercial radioimmunassay (Axis-Shield, Oslo, Norway). Inter- and intraassay CVs were < 8%.

Statistical Analysis

A Kolmogorov-Smirnov test was used to test for normal distribution. Vitamin B_{12}, MMA, holoTC, folate, and Hcy did not show a normal distribution. Hence, all laboratory variables are provided as median (25.-75. percentile). Anthropometrical data are given as mean ± standard deviation. Group comparison was done with a Mann-Whitney U-test. Furthermore, we performed a Spearman's correlation analysis with all biochemical markers.

Results

Athletes (runners and cyclists) had a mean age of 38 ± 7 y, a mean height of 178 ± 7 cm, and a mean weight of 72 ± 9 kg. The weekly training volume ranged between 5 to 10 h and regular training had continued over the last 7 ± 6 y. Training was not standardized. Controls had a mean age of 38 ± 9 y, a mean height of 176 ± 9 cm, and a mean weight of 73 ± 12 kg. All subjects had an omnivorous diet and did not use vitamin supplements. The descriptive statistics of all laboratory parameters are presented in Table 1. Median vitamin B_{12} and folate levels were comparable in athletes and controls, and ranged within the reference intervals of the applied assays (lower reference limit of folate: 5.38 ng/mL; reference interval of vitamin B_{12}: 211 to 911 pg/mL). These results indicate a comparable and sufficient micronutrient status in both groups. MMA and holoTC were, however, significantly higher in athletes than in controls. The median MMA level in athletes was close to the upper reference limit of 270 nmol/L.

Table 1 Descriptive Statistics and Group Comparison Between Athletes and Controls

Parameter	Controls (n = 46)	Athletes (n = 72)	P–value
Vitamin B_{12}	350 (255–428)	343 (285–431)	0.83
Holotranscobalamin II (pmol/L	55 (45–70)	67 (52–93)	0.004*
Methylmalonic acid (nmol/L)	175 (141–266)	242 (196–324)	< 0.001*
Folate (ngl/mL)	8.6 (6.1–15.1)	8.3 (6.1–10.9)	0.52
Homocystein (μmol/L)	10.8 (8.9–12.9)	9.2 (7.2–12.6)	0.06

Note. Results are given as median (25.–75. percentile); *P < 0.05.

Figure 1 depicts the individual vitamin B_{12}, holoTC, and MMA levels of all subjects investigated. Four athletes exhibited vitamin B_{12} serum levels below the lower reference limit and 4 athletes had lowered holoTC concentrations (lower reference limit: 35 pmol/L). One athlete with lowered holoTC had also a lowered vitamin B_{12}. Based on holoTC and vitamin B_{12} results, 7 athletes (10%) would have been classified as vitamin B_{12} deficient. Both markers, however, represent only the extracellular vitamin B_{12} status. By contrast, MMA, a marker of the functional intracellular vitamin B_{12} supply, was increased in 25 athletes (35%). Only one of those 25 athletes had a lowered vitamin B_{12} serum level. In controls, we found 2 subjects with lowered vitamin B_{12} and five with lowered holoTC levels. Altogether, we would have classified 7 controls (15%) as deficient for circulating vitamin B_{12}. Only 9 of 47 controls (19%) had elevated MMA levels, which is significantly less than in athletes. Considering folate levels, we found a comparable frequency of folate deficiencies (15%) in athletes (n = 10) and controls (n = 7). Furthermore, median Hcy, a cumulative marker of the methionine metabolism, was clearly below the upper reference limit in both groups (upper reference limit < 12μmol/L), and tended to be somewhat lower in athletes than in controls (about 15%; P = 0.06).

As expected, in controls there were significant correlations between vitamin B_{12} and MMA (r = –0.38, P = 0.008), vitamin B_{12}, and holoTC (r = 0.51,

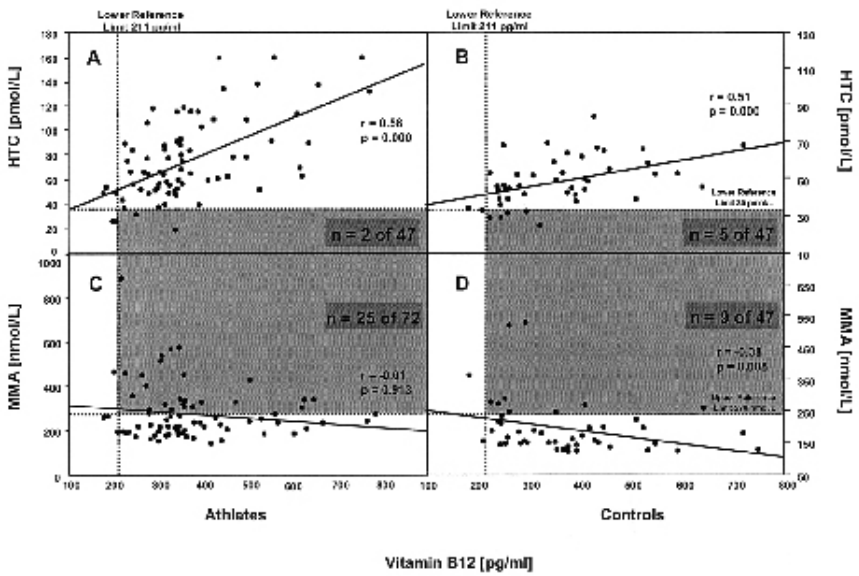

Figure 1 — Scatterplots of vitamin B_{12}, holoTC, and MMA results of all subjects with the corresponding regression line. Plots A and C display the data of athletes and plots B and D depict the data of controls. Each plot contains the corresponding correlation coefficient and the *P*-value. Furthermore, the relevant reference limits of vitamin B_{12}, holoTC, and MMA are shown (dotted lines)

$P = 0.000$), MMA, and holoTC ($r = –0.36$, $P = 0.016$) as well as Hcy and folate ($r = –0.404$, $P = 0.006$). Furthermore, Hcy correlated with vitamin B_{12} and holo TC on a 10% level (Hcy vs. vitamin B_{12}: $r = –0.27$, $P = 0.075$; Hcy vs. holoTC: $r = 0.28$, $P = 0.067$).

In athletes, vitamin B_{12} correlated significantly with holoTC ($r = 0.58$, $P = 0.000$). Hcy correlated with folate ($r = –0.43$, $P = 0.000$), vitamin B_{12} ($r = –0.33$, $P = 0.006$), and holoTC ($r = –0.29$, $P = 0.017$). In contrast to controls, the correlations of vitamin B_{12} with MMA ($r = –0.01$, $P = 0.913$) and MMA with holoTC ($r = 0.01$, $P = 0.931$) were not present in athletes.

In a next step, we divided athletes and controls in tertiles of vitamin B_{12} and holoTC, and calculated the corresponding medians of MMA (Figure 2). In controls MMA levels decreased from the first to the third tertile independently if classification was done for vitamin B_{12} or holoTC. Athletes exhibited no such decrease.

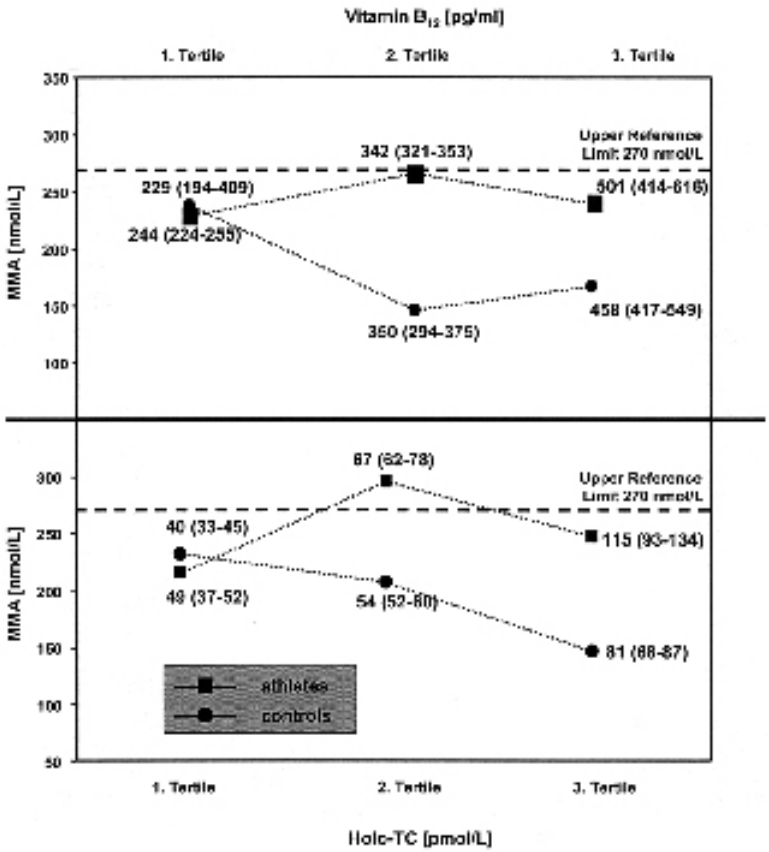

Figure 2 — Analysis of median MMA concentrations in tertiles of vitamin B_{12} and holoTC. The numbers beside each data point represent the median (25.-75. percentile) vitamin B_{12} or holoTC concentration in the corresponding tertile. The dashed lines display the upper reference limit of MMA.

Discussion

Athletes and controls had similar vitamin B_{12} and folate serum levels (within the reference intervals) indicating a comparable and sufficient status of these micronutrients. Analysis of Hcy, MMA, and holoTC suggest an altered vitamin B_{12} metabolism, however, but a normal folate status in athletes.

Median holoTC was significantly higher in athletes than in controls (Table 1). This finding was somewhat surprising, since serum vitamin B_{12} was comparable in both groups. Vitamin B_{12} measurement detects a group of chemically related molecules consisting of four pyrrol rings with a central cobalt atom. Only holoTC, however, which accounts for 6 to 20% of circulating vitamin B_{12}, can be utilized by all cells (12). Recent studies have demonstrated that measurement of holoTC represents better circulating vitamin B_{12} than common vitamin B_{12} assays (5, 13, 14, 21). Since athletes had higher holoTC levels, we expected a better cellular vitamin B_{12} supply, expressed by lower MMA levels. But we found significantly higher MMA levels in athletes than in controls. Median MMA in athletes was close to the upper reference limit, suggesting a critical intracellular vitamin B_{12} supply. Correlation analysis revealed a significant association between vitamin B_{12} and holoTC, which was comparable in both groups. In addition, in controls we found significant correlations between MMA, vitamin B_{12}, and holoTC. These results are in accordance with previous results by others and ourselves (11, 15, 16). In athletes, however, the correlations of vitamin B_{12} and holoTC (markers of extracellular vitamin B_{12} supply) with MMA (marker of intracellular vitamin B_{12} supply) were not present, suggesting a disturbed cellular vitamin B_{12} metabolism.

What might be the reason for the discrepancy between MMA and holoTC in athletes? The main difference between athletes and controls was the regular endurance training. Endurance exercise is known to cause changes in plasma volume, which might affect the serum concentration of all molecules which can not freely pass the vessel wall (7). MMA, however, represents a very small molecule that is not concentrated or diluted by changes in plasma volume. Consequently, hemoconcentration as the underlying cause for higher MMA levels in athletes can be ruled out. An exercise-induced stimulation of β-oxidation in athletes might be another hypothetical explanation for the discrepancy between MMA and holoTC. Propionic acid, a metabolite in the β-oxidation cascade, is transformed into propionyl-CoA, representing the direct precursor of MMA. Therefore, a stimulated β-oxidation in athletes might result in an increased formation of MMA (independent of vitamin B_{12}). We have demonstrated, however, that endurance exercise causes only a brief (reversible within a few hours) transient MMA increase (unpublished data). In the present study, blood sampling was performed at rest (≥ 8 h after the last training session) and 1 to 12 h before a long-distance race (marathon running or a 110 km mountain bike race). Accordingly, the last training session before blood sampling was short and of low intensity. That is why a stimulated β-oxidation as an explanation for higher MMA levels in athletes seems improbable. Elevated MMA levels in the presence of normal vitamin B_{12} and holoTC levels can also be due to intestinal bacterial overgrowth (20). It has been shown that intestinal bacteria are able to synthesize MMA, which appears in the circulation. A high prevalence of bacterial overgrowth in athletes has never been described, however, and would probably have been recognized by clinical symptoms.

Compared to inactive controls, athletes are exposed to oxidative stress, inducing multiple modifications of cell physiology (6, 17, 18). Two previous studies on vegetarians and neurological patients have shown that oxidative stress could be related to a disturbed vitamin B_{12} metabolism (16, 22). McCaddon et al. reported that in patients with Alzheimer's disease oxidative stress might hamper the intracellular reduction of vitamin B_{12} to its metabolically active form (22). Herrmann et al. found significantly higher MMA levels and a reduced antioxidative status in vegetarians than in omnivorous controls (16). Moreover, in vegetarians, the antioxidative status and MMA were significantly correlated. Based on these findings, we hypothesize that oxidative stress might be a relevant causative factor for the altered vitamin B_{12} metabolism in athletes. We did not, however, quantify oxidative stress and, therefore, this hypothesis is highly speculative and remains to be proven.

Given that vitamin B_{12} deficiencies are involved in the pathogenesis of various diseases, such as anemia and neurodegenerative disorders (11), it would be interesting to know whether vitamin B_{12} supplementation can improve intracellular vitamin B_{12} status in athletes. Since our subjects did not use supplements, we cannot answer this question. A hint, however, can be obtained by the analysis of MMA when subjects are divided in tertiles of vitamin B_{12} or holoTC. As expected, controls exhibited a significant decline of MMA from the first to the third tertile. In contrast, athletes showed no decline of MMA. Even in the third tertile (vitamin B_{12}:501 pg/mL; holoTC: 115 pmol/L) MMA did not fall below 250 nmol/L, which indicates a critical intracellular B_{12} status. Because of the possibly disturbed cellular vitamin B_{12} metabolism it is questionable whether athletes would benefit from an oral vitamin B_{12} substitution. Only an intervention trial, however, can clarify this question.

The second vitamin investigated was folate. Most folate assays detect 5-methyltetrahydrofolate, the major form in human serum, which correlates well with the intracellular folate supply. The methyl group of 5-methyltetrahydrofolate is intracellularly transferred onto Hcy to form methionine (remethylation). Folate deficiency leads to an inhibition of this reaction with a subsequent accumulation of Hcy (14). Athletes and controls exhibited comparable folate levels within the reference interval. Moreover, athletes had slightly lower Hcy levels than controls, indicating a sufficient intracellular folate status. The correlation between Hcy and folate was similar in athletes and controls. These results demonstrate that folate metabolism is comparable in the 2 groups.

Lastly, our data suggest a disturbed cellular vitamin B_{12} utilization among recreational endurances athletes. The underlying mechanism of the disturbed vitamin B_{12} metabolism is not understood and needs clarification. In contrast, folate status is normal in athletes and controls. Therefore, a general recommendation to substitute folate in athletes is not justified.

References

1. Allen, R.H., S.P. Stabler, J. Lindenbaum. Serum betaine, N,N-dimethylglycine and N-methylglycine levels in patients with cobalamin and folate deficiency and related inborn errors of metabolism. *Metabolism*. 42:1448-1460, 1993.
2. Bjorke Monsen, A.L., and P.M. Ueland. Homocysteine and methylmalonic acid in diagnosis and risk assessment from infancy to adolescence. *Am. J. Clin. Nutr*. 78:7-21, 2003.

3. Bottiglieri, T., and K. Hyland. S-adenosylmethionine levels in psychiatric and neurological disorders: a review. *Acta Neurol. Scand.* 154:19-26, 1994.
4. Chiang, P.K., R.K. Gordon, J. Tal, G.C. Zeng, B.P. Doctor, K. Pardhasaradhi, and P.P. McCann. S-Adenosylmethionine and methylation. *FASEB J.* 10:471-480, 1996.
5. Clarke, R., H. Refsum, J. Birks, J.G. Evans, C. Johnston, P. Sherliker et al. Screening for vitamin B-12 and folate deficiency in older persons. *Am. J. Clin. Nutr.* 77:1241-1247, 2003.
6. Cooper, C.E., N.B. Vollaard, T. Choueiri, and M.T. Wilson. Exercise, free radicals and oxidative stress. *Biochem. Soc. Trans.* 30:280-285, 2002.
7. Dill, D.B., and D.L. Costill. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J. Appl. Physiol.* 37:247-248, 1974.
8. Economos, C.D., S.S. Bortz, and M.E. Nelson. Nutritional practices of elite athletes. Practical recommendations. *Sports Med.* 16:381-399, 1993.
9. Faber, M., and A.J. Benade. Mineral and vitamin intake in field athletes (discus-, hammer-, javelin-throwers and shotputters). *Int. J. Sports Med.* 12:324-327, 1991.
10. Finkelstein, J.D. The metabolism of homocysteine: pathways and regulation. *Eur. J. Pediatr.* 157 Suppl 2:40-44, 1998.
11. Herbert V (1994): Staging vitamin B-12 (cobalamin) status in vegetarians. *Am. J. Clin. Nutr.* 59,1213-22.
12. Herbert, V., W. Fong, V. Gulle, and T. Stopler. Low holotranscobalamin II is the earliest serum marker for subnormal vitamin B12 (cobalamin) absorption in patients with AIDS. *Am. J. Hematol.* 34:132-139, 1990.
13. Herrmann, W., R. Obeid, H. Schorr, and J. Geisel. Functional vitamin B12 deficiency and determination of holotranscobalamin in populations at risk. *Clin. Chem. Lab. Med.* 41,1478-88, 2003.
14. Herrmann, W., H. Schorr, R. Obeid, and J. Geisel. Vitamin B-12 status, particularly holotranscobalamin II and methylmalonic acid concentrations, and hyperhomocysteinemia in vegetarians. *Am. J. Clin. Nutr.* 78,131-136, 2003.
15. Herrmann, W., H. Schorr, M. Bodis, J.P. Knapp, A. Muller, A. Stein, and J. Geisel. Role of homocysteine, cystathionine and methylmalonic acid measurement for diagnosis of vitamin deficiency in high-aged subjects. *Eur. J. Clin. Invest.* 30:1083-1089, 2000.
16. Herrmann, W., H. Schorr, K. Purschwitz, F. Rassoul, and V. Richter. Total homocysteine, vitamin B(12), and total antioxidant status in vegetarians. *Clin. Chem.* 47,1094-1101, 2001.
17. Ji, L.L. Antioxidants and oxidative stress in exercise. *Proc. Soc. Exp. Biol. Med.* 222: 283-292, 1999.
18. Konig, D., K.H. Wagner, I. Elmadfa, and A. Berg. Exercise and oxidative stress: significance of antioxidants with reference to inflammatory, muscular, and systemic stress. *Exerc. Immunol. Rev.* 7:108-133, 2001.
19. Krumbach, C.J., D.R. Ellis, and J.A. Driskell. A report of vitamin and mineral supplement use among university athletes in a division I institution. *Int. J. Sport Nutr.* 9:416-425, 1999.
20. Lindenbaum, J., D.G. Savage, S.P. Stabler, and R.H. Allen. Diagnosis of cobalamin deficiency: II. Relative sensitivities of serum cobalamin, methylmalonic acid, and total homocysteine concentrations. *Am. J. Hematol.* 34:99-107, 1990.
21. Lloyd-Wright, Z., A.M. Hvas, T.A. Sanders, and E. Nexo. Holotranscobalamin as an indicator of dietary vitamin b(12) deficiency. *Clin. Chem.* 49:2076-2078.

22. McCaddon, A., B. Regland, P. Hudson, and G. Davies. Functional vitamin B(12) deficiency and Alzheimer disease. *Neurology*. 58:1395-1399, 2002.
23. Schroder, H., E. Navarro, J. Mora, J. Seco, J.M. Torregrosa, and A. Tramullas. The type, amount, frequency and timing of dietary supplement use by elite players in the First Spanish Basketball League. *J. Sports Sci*. 20:353-358, 2002.
24. Seetharam, B., S. Bose, and N. Li. Cellular import of cobalamin (Vitamin B-12). *J. Nutr.*129:1761-1764, 1999.
25. Selhub, J. Folate, vitamin B12 and vitamin B6 and one carbon metabolism. *J. Nutr. Health Aging*. 6:39-42, 2002.
26. Singh, A., P. Evans, K.L. Gallagher, and P.A. Deuster. Dietary intakes and biochemical profiles of nutritional status of ultramarathoners. *Med. Sci. Sports Exerc*. 25:328-334, 1993.
27. Stabler, S.P., J. Lindenbaum, and R.H. Allen. The use of homocysteine and other metabolites in the specific diagnosis of vitamin B-12 deficiency. *J. Nutr*. 126:1266S-1272S, 1996.
28. Stead, L.M., K.P. Au, R.L. Jacobs, M.E. Brosnan, and J.T. Brosnan JT. Methylation demand and homocysteine metabolism: effects of dietary provision of creatine and guanidinoacetate. *Am. J. Physiol. Endocrinol. Metab*. 281:E1095-E1100, 2001.
29. Van der Beek, E.J. Vitamins and endurance training. Food for running or faddish claims? *Sports Med*. 2:175-197, 1985.