

Plasma B-6 Vitamer Changes Following a 50-km Ultramarathon

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The purpose of this study was to measure plasma B-6 vitamers, and other factors which may affect the plasma concentrations of these vitamers under extreme physical conditions. Blood samples were drawn from 8 men and 3 women (43.7 ± 8.6 years) 30 min prior to the start of a 50-km ultramarathon race (pre), and at 5 (PST) and 60 (PST60) min post race. HPLC was used to measure plasma pyridoxal 5'-phosphate (PLP), pyridoxal (PL), pyridoxine (PN), and 4-pyridoxic acid (4-PA). Plasma glucose, albumin, lactate, and alkaline phosphatase activity, as well as hematocrit, and hemoglobin levels were measured. Food and liquid intake was assessed during the run. There was a significant ($p < .001$) decrease in the plasma PLP concentration between pre and PST, with a mean decrease of 12.9 ± 8.8 nmol/L (31% decrease). At PST60, there was a further decrease in plasma PLP concentration bringing the total decrease to 17.9 nmol/L (44%). The plasma TB6 concentration also decreased after the run, but the mean decrease was only 13.5 nmol/L (pre to PST60). PL increased 25% after the run, and did not change further at PST60. The mean plasma 4-PA concentration increased 21% post run and decreased to just below the pre-run value 1 hr post race. The plasma PLP decrease measured in the current study is not consistent with what has previously been reported during shorter length endurance studies.

Key Words: pyridoxal 5'-phosphate, pyridoxal, 4-pyridoxic acid, exercise, lactate

Endurance athletes create different physiological conditions in their bodies, compared to sedentary individuals, due to the amount of physical stress they endure while training and competing. During sedentary and exercising conditions, the dominant sources of energy supplied to the working muscles are free fatty acids and carbohydrates. During high endurance activities, such as running, additional sources of energy become required. As the duration of exercise increases, a large percentage of this energy comes from the formation of glucose from non-glucose substrates (27), which is termed *gluconeogenesis*. This energy forming process can include the use of lactate, pyruvate, or glycerol, as well as the deamination of amino acids so that their carbon skeletons are made available for gluconeogenesis. Another source of

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energy is the breakdown of stored muscle and liver glycogen to glucose, which is termed *glycogenolysis*.

Both glycogenolysis and gluconeogenesis from amino acid deamination require vitamin B-6 as its coenzyme form, pyridoxal 5'-phosphate (15). As the length of an endurance event increases, there is an increased reliance on pyridoxal 5'-phosphate (PLP), requiring enzymes in the working muscles and the liver due to their role in these energy producing pathways (16).

There have been relatively few studies of the effect of high endurance exercise on vitamin B-6 metabolism. Previous researchers have found plasma PLP concentration increases during exercise, and remains elevated throughout the exercise bout. This has been documented in short-term intense exercise (<30 min, 80% $\dot{V}O_{2max}$) (20, 22), long-term moderate exercise (50–120 min, 60–75% $\dot{V}O_{2max}$; ref. 12, 13), and after a marathon (28). Rokitzki et al. (28) have been the only investigators to measure serum vitamin B-6 changes after an actual endurance event longer than 4,500 m (20). They observed a significant increase in mean vitamin B-6 serum concentrations (individual metabolite concentrations not specified) of 53 nmol/L (assuming this is PLP), immediately after the race ($p < .001$). The average age of their subjects was 35.6 ± 9.8 years, with their personal best marathon times ranging from 2.5–3 hr. Compared to the study by Rokitzki et al. (28), the group studied here was slightly older, the course was longer (50 km) and included a very mountainous terrain with a total elevation gain and loss of 3.66 km (12,000 feet). Added up, these conditions created a greater metabolic challenge on the human body than previously studied.

Ultramarathons of this length, 50–100 km (9, 25) and even longer (1), are becoming increasingly popular. Over the last 10 years, physiological information on this group of athletes has started to become available, but there has been no information published on the vitamin B-6 concentration changes after an ultramarathon. An extreme metabolic change, analogous to acute starvation, is created during an exercise session such as this, thus creating an environment in which these changes can be evaluated.

The aim of this study will be to measure the acute changes in plasma B-6 vitamer concentrations after an ultramarathon (50 km) in order to increase our knowledge of what may be physiologically occurring in exercising individuals under these extreme conditions. Based on the previous work in this area, and the extreme demands on fuel substrate utilization, the expected outcome is that the plasma PLP levels will increase after the exercise event, and return to baseline levels 1 hr post race.

Methods

Subjects

Eleven trained adults (3 female, 8 male) participated in this study after giving informed written consent. The subjects' physical characteristics are shown in Table 1. The protocol was approved by the Institutional Review Board (IRB) for the protection of human subjects at Oregon State University. The subjects were recruited through a mailed flyer from applicants who were planning to participate in a 50-km (31 mile) ultramarathon, which was held independent of the University and the researchers. They were non-smokers who were asked to forgo supplements containing vitamin B-6 for 48 hr prior to the day of the race.

Experimental Protocol

A breakfast meal was provided to the subjects. The breakfast consisted of an 8-oz. glass of orange juice and a bagel with jelly on it. The meal was ~500 calories, and contained 0.47 mg of vitamin B-6. The pre-race meal was to be consumed 3 hr prior to the start of the race. Certain foods and liquids were available to the subjects during the race (Table 2). Water was available ad libitum on the course. The foods were chosen for their low vitamin B-6 content as well as their availability for easy access and consumption. The foods consumed by each subject were recorded at all but the first aid station by course attendants. Energy and nutrient consumption were calculated using a computerized dietary analysis program (Food Processor II, ESHA Research, Salem, OR). The information is presented in Table 3.

Table 1 Physical Characteristics of the Subjects ($n = 11$)

Variable	Mean	SD
Age (years)	43.7	8.6
Height (cm)	173	9
Pre-race weight (kg)	71.9	9.3
Post-race weight (kg)	70.3	9.1
BMI (kg/m ²)	24.0	2.0
Average training distance (miles/week)	60	—

Table 2 Foods Available for Breakfast, and During the Race and Their Energy and Vitamin B-6 Values

Food	Serving size (g)	B-6 (mg)	Energy (kcal)
Breakfast			
Bagel	110	0.364	300
Orange juice	249	0.100	112
Jelly	30	0.006	81
During the race			
Oreo® cookies	33	0.030	160
Hydra® fuel	240	—	200
Orange, slices	17	0.010	8
Gu®	25	—	100
Pretzels	10	0.012	38
Cola	240	—	100

Note. Oreo is a trademark of Nabisco Foods, East Hanover, NJ. Hydra Fuel is a trademark of TwinLab, Ronkonoma, NY. Gu is a trademark of Sports Street Marketing, Berkeley, CA.

Table 3 Nutrient Composition of the Foods Selected at the Aid Stations (Mean \pm SD)

Aid station #	B6 (mg)	Protein (g)	CHO (g)	Total energy (kcal)
1	n.a.	n.a.	n.a.	n.a.
2	0.05 \pm 0.06	0.87 \pm 1.24	35.7 \pm 16.2	52 \pm 70
3	0.04 \pm 0.05	0.85 \pm 1.07	58.8 \pm 52.8	36 \pm 237
4	0.05 \pm 0.06	0.61 \pm 0.86	70.1 \pm 40.1	87 \pm 155
5	0.02 \pm 0.06	0.31 \pm 0.64	40.0 \pm 32.0	65 \pm 133
Total	0.16 \pm 0.14	2.64 \pm 1.68	198.9 \pm 55.7	39 \pm 230

Of the 11 subjects, 6 were from the Corvallis area. To assess the pre-race plasma PLP concentration, an overnight 12-hr fasting blood sample of 20 ml was obtained from the 6 local subjects 48 hr prior to the start of the race. To measure post-race plasma differences, 20-ml blood samples were collected 30 min prior to the start of the race (PRE), and at 5 (PST) and 60 (PST 60) min post race, from all 11 subjects. Between the PST and PST60 blood draws, the subjects were allowed to drink up to 500 ml of water. No foods were consumed during this period. Blood samples were drawn from the median cubital vein with subjects in the seated position (after 5 min rest). The blood was drawn by a trained phlebotomist in a screened off section of a facility that was adjacent to the start/finish line. Blood samples were collected in heparinized tubes, mixed slightly, and immediately put on ice in a closed container. The samples were transported to the laboratory once an hour. All samples were centrifuged at $1800 \times g$ for 15 min at 4 °C. Plasma was collected and stored frozen (-40 °C) for subsequent analysis.

Laboratory Analyses

Pyridoxal 5'-phosphate (PLP), pyridoxal (PL), 4-pyridoxic acid (4-PA), and pyridoxine (PN) concentrations were analyzed by high performance liquid chromatography according to a modified method of Sharma and Dakshinamurti (30). Chromatographic analyses were performed with a Shimadzu controller (model SCL-10H); two Shimadzu pumps (model LC-10AD); Shimadzu 250 μ L injection loop; a Rainin C₁₈ ion-pair analytical column (model 86200E3, 3- μ m particle size, 4.6 \times 100 mm, Rainin Instrument, Emeryville, CA). Fluorescence was measured with a Shimadzu Spectrofluorometric Detector (model RF-10A) at an excitation wavelength of 330 nm and 400 nm emission. The gradient system used was 0.033 M phosphate/8mM octane sulfonic acid (for the first mobile phase) and 0.033 M phosphate/isopropanol (18%, v:v) for the second mobile phase, both at a pH of 2.3. The flow rate was 1.0 ml/min. The post column reagent (1.0 g/L sodium bisulfite in 1 M KH₂PO₄, pH 7.5) was added to the column eluate at 0.2 ml/min. Each subject's samples were analyzed in one assay to minimize the effect of inter-assay variation. Inter-assay variation of a control sample ($n = 8$) was 14%. Glucose was analyzed in duplicate using a modified procedure of Trinder (33) on a Technicon Autoanalyzer. Lactate was determined by

the method described by Hohorst (14). Alkaline phosphatase activity was determined by a colorimetric assay (29). All samples were run in duplicate. Albumin was determined using a method by Slater et al. (32). Immediately upon arrival of the samples at the lab, hemoglobin (Hb) and hematocrit (Hct) values were determined. Hb and Hct values were used to calculate changes in plasma volume, as described by Dill and Costill (8).

Statistical Analysis

Data are expressed as means \pm SD. Because the data are repeated measures on the same subjects, the means for all variables in Tables 4 and 5 were analyzed by repeated measures analysis of variance (ANOVA) and significant differences determined. For those dependent variables with significant *F*-ratios, Scheffe's post

Table 4 Mean Plasma B-6 Vitamer Concentrations Before and After a 50-km Ultramarathon Run, as Measured by HPLC (Mean \pm SD)

Variable	Pre-race	Post-race	Post 1 hr	Normal values ¹
PLP (nmol/L)	41.1 \pm 14.2 ^{ab}	28.2 \pm 10.8 ^a	23.2 \pm 9.9 ^b	>30 nmol/L
PL (nmol/L)	18.6 \pm 8.0	23.3 \pm 14.0	23.3 \pm 11.6	NV ³
4-PA (nmol/L)	25.5 \pm 7.6	30.8 \pm 8.6 ^c	23.4 \pm 5.4 ^c	NV ³
PN (nmol/L)	12.6 \pm 2.4	13.2 \pm 3.3	12.3 \pm 2.8	NV ³
TB-6 (nmol/L) ²	72.3	64.7	58.8	>40 nmol/L

Note. In a given row those that share the same letter are significantly different at: ^{ab}($p < .001$); ^c($p < .05$).

¹Data taken from reference (17).

²Total B-6 values in this table were calculated by adding the PLP, PL, and PN values together.

³NV = no value established, limited data are available.

Table 5 Mean Plasma Glucose, Lactate, and Albumin Concentration and Alkaline Phosphatase Activity Before and After a 50-km Ultramarathon (Mean \pm SD)

Variable	Pre-race	Post-race	Post 1 hr
Glucose (mmol/L)	4.60 \pm 1.22	4.77 \pm 0.83	4.44 \pm 0.83
Lactate (mmol/L)	1.86 \pm 0.44 ^a	2.52 \pm 0.66 ^a	2.04 \pm 0.92
Albumin (g/L)	49.3 \pm 3.8	52.1 \pm 4.0	52.5 \pm 3.9
Alk phos (u/L)	27.2 \pm 6.8	27.9 \pm 6.8	27.7 \pm 7.0

^aIn a given row those that share the same letter are significantly different at ($p < .05$)

hoc test was used to see where those differences lie. Differences were considered to be significant at $p < .05$. Pearson product-moment correlation coefficient (r) was used to measure associations between variables, including plasma B-6 vitamers and plasma alkaline phosphatase (which reflects hydrolysis of PLP) activity, plasma glucose and lactate (as measures of fuel substrates/metabolites), as well as race time and plasma PLP concentration change (pre/PST).

Results

The mean B-6 vitamer concentrations as determined by HPLC are given in Table 4. Mean plasma PLP concentration significantly ($p < .001$) decreased by 12.9 ± 8.8 nmol/L (31% decrease pre to post race). One hour post race, the mean value decreased further by 5 nmol/L (post to post 1 hr), bringing the total decrease to 17.9 nmol/L (44% decrease from pre to 1 hr post race).

The mean plasma PL post race value increased 25%, but this change was not found to be significant. There was no further change in PL 1 hr post race. The mean plasma PN change was very small after the race and was not found to be significant. Plasma 4-PA was measured, since it is the metabolic end-product of vitamin B-6 and is not metabolized back to PL or PLP. Plasma 4-PA is produced in the liver, transported in the blood, cleared through the kidneys, and excreted in the urine. The mean plasma 4PA concentration increased 21% post race, but was not found to be significant ($p > .10$), and then came back down to just below the pre-race value 1 hr post race. However, there was a significant difference in 4-PA concentration between PST and PST60 ($p < .05$).

The pre-race mean plasma PLP concentration (normal value > 30 nmol/L) does indicate adequate vitamin B-6 status for the group as reported by Leklem (17). For the 6 subjects who had a 48-hr fasting blood sample drawn, the plasma PLP concentrations correlated positively with the pre-race values ($p < .01$), indicating that the pre-race plasma PLP concentrations were a good representation of fasting concentrations.

The acute ingestion of dietary carbohydrate has been shown to affect plasma vitamin B-6 levels (13, 19). In addition to evaluating the effect of exercise per se, the plasma glucose levels were assayed to also measure the effect of the small amounts of food and carbohydrate drink consumed during the race, the majority of which came from aid station four, 8.2 miles from the finish line. These values are presented in Table 5. The mean plasma glucose concentration was only 2% higher after the race and returned to a level that was below the pre race baseline at 1 hr post race. Plasma alkaline phosphatase activity and albumin concentrations were measured, and no significant changes were found (Table 5). The plasma alkaline phosphatase activity (alk phos) and albumin concentrations were compared with the plasma PLP concentrations (Table 6) to look for variable associations to help explain the PLP changes. Neither of the correlations were found to be significant. The mean plasma lactate concentration was found to increase by 26% from pre to PST ($p < .05$). A larger increase was found than what was expected. The significant increase could be due to a strong push at the end of the race to finish strong in front of the crowd. The relationship between plasma PLP concentration and plasma lactate concentration was also examined (Table 6). As with albumin and alkaline phosphatase activity, no significant correlation was found.

Table 6 Correlation Coefficients for the Plasma PLP Concentration Levels vs. the Plasma Albumin, and Lactate Concentrations, As Well As Alkaline Phosphatase Activity

Factors	Pre	Post	Post1 hr
Alk phos/PLP	-0.4976	-0.4305	-0.3150
Albumin/PLP	0.3535	0.3370	0.2671
Lactate/PLP	-0.2152	-0.3646	-0.2794

Mean hemoglobin and hematocrit values were used to calculate plasma volume changes by the method of Dill and Costill (8), since large shifts in plasma volume could affect the plasma B-6 vitamers concentrations. The mean plasma volume changes were -1.1 ± 5.3 (pre vs. PST), -1.3 ± 7.0 (pre vs. PST60), and 0.6 ± 6.5 (PST vs. PST60; mean \pm SD). Individual subject changes ranged from -13% to 14% , but due to some of the subjects having an increase and some having a decrease, the net plasma volume change was not significant for any of the means.

The subjects were asked to forgo supplement usage 48 hr prior to the day of the race, as well as on the day of the race. Prior to this period, 2 of the subjects supplemented with a multivitamin, and 2 were taking vitamin E. Vitamin C was the most common supplement used by the subjects. Eight of 11 subjects supplemented with approximately 1,000 mg of vitamin C a day. Shultz and Leklem (31) investigated the influence of ascorbic acid (AA) on the metabolism of vitamin B-6. It was concluded that short term AA supplementation did not alter vitamin B-6 metabolism. The reasons for the high incidence of vitamin C supplementation among this group may be the result of reports discussing its possible use as an antioxidant to aid in reducing the incidence of upper-respiratory-tract infections (24, 26), as well as its use in the formation of collagen. The subjects had a mean race completion time of 375.8 ± 47.6 min. Because of PLP being a coenzyme for glycogen phosphorylase and several reactions involved in gluconeogenesis, correlation of pre-race PLP concentration with race time was done to evaluate if a low vitamin B-6 status was associated with poor race performance. A significant correlation was not found, nor was a significant correlation found between plasma PLP concentration change (pre/PST) and individual race times.

Discussion

The significant decrease ($p < .001$) in plasma PLP levels of the subjects in this study are in contrast to previous studies (12, 13, 20, 22, 28) that have all reported an increase in plasma PLP after exercise. The large decrease in plasma PLP in this study appears to be independent of plasma lactate concentration, alkaline phosphatase activity, plasma glucose concentration, and to a lesser degree, albumin levels. The extreme physiological conditions experienced by the subjects during the 50-km race, such as the duration of the event, and the elevation gain and loss, may have affected the plasma PLP concentrations due to the need for PLP in reactions

involving non-primary fuel substrates (such as certain amino acids and pyruvate), during the later stages of the race.

One of the first studies to look at the plasma vitamin B-6 and PLP concentrations following an acute bout of endurance exercise was performed by Leklem and Shultz (20). Seven trained male adolescents had blood drawn before and immediately after a 4,500-m run on three separate occasions during their competitive season. The subjects had a significant mean increase in plasma PLP concentration after all three of the trials as well as collectively ($p < .01$). Similarly, the mean plasma total vitamin B-6 levels were also found to increase significantly ($p < .05$). Subsequently, there have been several studies conducted to look at these changes under different exercise conditions, as well as attempt to answer the question, as to why these changes occur.

Several studies have looked at the plasma PLP and total vitamin B-6 concentration changes using a cycle ergometer (7, 12, 22). These authors reported significant plasma PLP concentration increases (ranging from 8–13 nmol/L) after a short term intense exercise bout (<30 min, $\geq 80\%$ $\dot{V}O_{2max}$; ref. 7, 22), as well as after a slightly longer moderate exercise bout (50 min, 60 – 75% $\dot{V}O_{2max}$; ref. 7), similar to the early work by Leklem and Shultz (8–18 nmol/L increase; ref. 20). In contrast, Crozier et al. (7) reported that a large majority (79%) of the plasma PLP concentration increase occurred within 5 min after the onset of exercise. A possible hypothesis for this observation will be discussed in greater detail later. Manore et al. (22) measured the plasma PLP and plasma vitamin B-6 concentrations at 30 and 60 min post exercise. The plasma PLP and vitamin B-6 concentrations increased significantly post exercise, neared pre-exercise values at 30 min post exercise, and dropped slightly below the pre-exercise values at 60 min post exercise. The rise and fall of plasma PLP after exercise has since been reported by others (13, 28), confirming the work by Manore et al. (22). As mentioned earlier, the plasma PLP and total vitamin B-6 concentration decrease found in the current study immediately post exercise is in contrast to all of the shorter duration exercise studies mentioned above.

Prior to the current study, only two groups have examined the plasma PLP and total vitamin B-6 concentration changes using slightly longer running distances (13,28). Hofmann et al. (13) measured the plasma PLP concentration changes in 6 male subjects after a 2-hr treadmill run, and Rokitzki et al. (28) measured serum vitamin B-6, as well as whole blood B-6 concentration in 13 male athletes after a marathon. Once again the findings were similar to the earlier findings by Leklem and Shultz (20). In the study conducted by Hofmann et al. (13) the plasma PLP changes were measured in runners ingesting either water or a glucose polymer solution during exercise. The mean nmol/L increase in plasma PLP from pre to post run was 27.7 for the water group, and 28.8 for the glucose polymer group. In the study conducted by Rokitzki et al. a mean increase of 53 nmol/L of serum vitamin B-6 was observed. To see an increase in the plasma similar to the findings of Hofmann et al. approximately 1.36 mg of vitamin B-6 would need to be added to the diet (11). Similarly, it appears that twice that amount would need to be added to the diet to see the change measured by Rokitzki et al. In contrast to the previously mentioned studies, the overall mean plasma decrease of 17.9 nmol/L measured in the current study would require approximately 0.66 mg of vitamin B-6 to be withheld from the diet (11) to achieve the same affect. This is a very dramatic shift, especially when viewing prior research.

Based on the work of these studies several researchers have put forth three independent hypotheses to account for the plasma PLP changes that occur during endurance exercise (7, 13, 20). Leklem and Shultz (20) have suggested that the release of PLP into the plasma is a homeostatic control mechanism. The authors hypothesis is based on the work by Black et al. (2, 3), whose group showed that vitamin B-6 is stored in rat muscle coupled to glycogen phosphorylase. The activity of glycogen phosphorylase changed in response to energy restriction (3). Although the liver is the primary source for PLP under normal physiological conditions, Leklem and Shultz have hypothesized that during exercise the muscle may become the primary plasma PLP source. As the duration of exercise increases and the liver metabolism is directed toward gluconeogenesis, the liver PLP requirement increases due to its use as a coenzyme for amino acid transaminations (15). This theory to describe the plasma PLP change has been termed the PLP-to-liver hypothesis. In contrast, Hofmann et al. (13) have suggested that increased plasma PLP is destined for use in skeletal muscle, which has been termed the PLP-to-muscle hypothesis. In their discussion the investigators mention several facts to support their theory. The first is a review of work done by Bosron et al. (4) that showed that the PLP located in the cytosolic fraction of the rat liver is depleted preferentially under conditions of deficiency. Also, Hoffman et al. (13) suggested that since the serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALK) were all found to be within normal ranges, the increase in plasma PLP concentration could not be due to tissue injury.

As mentioned earlier several enzymes required for fuel substrate usage are PLP dependent (15). Hofmann et al. have suggested that even though glycogen phosphorylase is the body's primary storage site for vitamin B-6 (2, 3), exercise may create a unique condition wherein the storage function of glycogen phosphorylase is eliminated. The authors theorized that the glycogen phosphorylase molecule may require full saturation with PLP due to its increased activity. This along with the possibility of a lowered binding affinity caused by the lowered intracellular pH (23) could lead to a localized deficiency of PLP in the exercising muscle.

The muscle-to-liver, as well as the liver-to-muscle hypothesis might help explain some of what is actually occurring during exercise. But as of yet neither have been proven. In a very eloquent discussion by Crozier et al. (7), several key issues have been discussed as to why neither one of these scenarios may be what is actually occurring. Within their discussion, the authors also present a new theory as to why plasma PLP becomes elevated during endurance exercise. The researchers suggest that the rise in plasma PLP found during exercise may be due to a shift in PLP from the interstitial PLP pool into the plasma PLP pool. Their theory is based on the work of Coburn et al. (5,6), who suggested that plasma PLP is in equilibrium with the interstitial space because the distribution volume of PLP administered to goats and pigs was found to be significantly larger than the plasma volume. Albumin is known to be present in the interstitial fluid (10), but it is unclear as to the presence of vitamin B-6 in the interstitial fluid. Crozier et al. have suggested that PLP may be bound to albumin, as in the plasma (18), and during exercise a protein shift occurs from the interstitial space into the blood. In contrast, the subjects in the current study did experience a small increase in albumin concentration post exercise, but the mean plasma PLP concentration was found to decrease.

In the current study PL increased 4.7 nmol/L pre to PST, and the vitamin B-6 metabolic end product 4-PA increased 5.3 nmol/L from pre to PST. These changes in the B-6 vitamers reflect the conversion of PLP to PL. The dephosphorylated metabolite (PL) can then cross the hepatocyte membrane and thus be converted to 4-PA for excretion in the urine. Because the conversion of PLP to 4-PA, via PL, occurs in the liver as opposed to the muscle, the decrease in plasma PLP concentration and the significant increase in plasma 4-PA concentration PST versus PST60 supports the PLP-to-liver hypothesis, suggested by Leklem and Shultz (20). If this hypothesis is correct, increased PL conversion to 4-PA and its subsequent excretion via the kidney would lead to a loss of vitamin B-6 from the body under these exercise conditions. In contrast, two other studies (8, 17) do not appear to support the PLP-to-liver hypothesis as discussed below.

In a study by Crozier et al. (7) 79% of the 14% increase in plasma PLP in subjects exercised on a cycle ergometer for 20 and 30 min occurred within the first 5 min of exercise. A similar finding has also been discussed by Hofmann et al. (13) in a study in which 6 male subjects were exercised on a treadmill for 2 hr. Similar to the results reported by Crozier et al., an average of 63% of the rise in plasma PLP occurred within the first 30 min of exercise. The findings by these researchers do not appear to coincide with the postulation by Leklem's group, suggesting that the PLP increase in the plasma found during exercise is due to an adaptation in the fuel supply substrates.

An alternate explanation may be that during the early stages of exercise, metabolites, a change in intracellular pH, or hormones may stimulate the release of PLP from the storage pools in the muscle and/or liver into the plasma for subsequent use. If this were the case, then the shorter duration exercise studies may not have been long enough to see a decrease in plasma PLP concentration, as the now larger plasma PLP pool had not yet been sequestered for hepatic gluconeogenesis, as may have been the case in the current study.

An additional factor that may have influenced plasma PLP is the ingestion of carbohydrate rich foods during the race. There have been several studies examining the relationship between glucose ingestion and plasma vitamin B-6 concentration (13, 19, 21, 22). Ingestion of 1 g glucose/kg bodyweight (300 ml glucose solution) has been shown to decrease fasting PLP levels by a mean of 19.5% after a 5-hr period (19). In the same study, ingestion of 300 ml water resulted in a plasma PLP mean decrease of 6.0%, indicating that some of the decrease may have been due to fluid ingestion. Hofmann et al. (13) applied this experiment to exercise, and observed a different result. Six subjects were given 200 ml of water or a glucose polymer solution before, and every 30 min during a 2-hr treadmill run. The mean plasma PLP increase for both groups was 22–23% upon completion of the exercise bout. It appears that during exercise, the body's normal homeostatic mechanism, which deals with carbohydrate ingestion, is altered. The results from the study by Hofmann et al. suggests that the 33% plasma PLP decrease found in the current study is independent of the carbohydrate or liquid the subjects took in during the race. When viewing this data it is also important to keep in mind that the subjects consumed approximately 0.20 mg of vitamin B-6 during the race, a factor which would increase plasma PLP concentration.

Another area that must be discussed when looking at nutrient changes in the plasma during exercise are the plasma volume changes. The daytime ambient tem-

perature ranged from 10–15 °C, and it was drizzly and overcast, which made it somewhat easier to stay hydrated. Due to variable plasma volume changes (PVCs), the mean net changes were not found to be significantly different from zero, and thus the plasma B-6 vitamers levels in the current study were not adjusted for PVCs.

In the present study, the change in PLP (pre/post) correlated significantly to the pre-race values ($p < .02$). This negative correlation may be due to how tightly PLP is bound to albumin/other proteins, and subsequent to alkaline phosphatase hydrolysis.

This is the first study to look at the vitamin B-6 changes in an endurance event of this length. Manore and Leklem (22) reported plasma PLP concentrations that were below resting values 30 min post-exercise, but as of yet our study is the first to find plasma vitamin B-6 and PLP concentrations below resting concentration values immediately following an endurance exercise bout. Since the results are different from previous research in this area, new questions surrounding PLP and vitamin B-6 concentration levels may be posed. For example, are the muscle PLP pools affected after exercise? And if so, how much? More research is needed to understand the mechanism as to why there is increased conversion of PLP to 4-PA during long term endurance exercise and if this results in an increased need for vitamin B-6.

Conclusion

This study provides data on the plasma B-6 vitamers changes in a group of ultramarathon runners. The decrease in plasma PLP found upon completion of the 50-km race is contradictory to previously reported data, using shorter distances. No significant correlations were found between the plasma PLP changes and race time, glucose, albumin and lactate levels, or alkaline phosphatase activity. The decrease in plasma PLP found in this study, as opposed to the increase found in previous studies, may be due to the length of the course and/or the added stress placed upon the body from a course that included 3.66 km of total elevation gain and loss. We have also hypothesized that during endurance exercise the body's homeostatic control mechanism may be to release PLP during the early stages of exercise for subsequent usage by the liver and/or the muscle. If the PLP that has been shifted into the plasma is not used during the exercise period, then it is taken back up into storage pools.

Based on the design and results of this study, it is not possible to definitively suggest that vitamin B-6 is being metabolized at an increased rate during exercise in this group of athletes. To better understand what is physiologically happening to vitamin B-6 metabolism during endurance events of this length and longer, future studies should include a controlled diet, determination of urinary 4-PA, and if possible muscle biopsies. These are the studies that are needed to answer the long standing question of how and why these shifts occur during exercise.

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