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Changes in plasma and urinary taurine and amino acids in runners immediately and 24 h after a marathon

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Summary. Changes in urinary and plasma taurine and amino acids have been evaluated in trained runners competing in the Rotterdam Marathon, 1998, both immediately after completing the event and 24 h after recovery. There were significant changes in the urinary amino acids excretion, the majority showing a significant decrease both immediately at the completion of the Marathon and after 24 h recovery. In contrast urinary taurine excretion increased immediately post Marathon, although not significantly as the range of results was wide. Such changes in urinary taurine correlated with percentage changes in plasma creatine kinase both immediately post race, ($r = 0.972$, $P < 0.001$), and 24 h later ($r = 0.872$, $P < 0.001$), possibly indicating that the source of the taurine was muscle. Significant correlations between the individual values for urinary and plasma amino acids in all of the athletes were calculated for taurine ($r = 0.528$), glycine ($r = 0.853$), threonine ($r = 0.749$), alanine ($r = 0.747$), serine ($r = 0.620$), glutamine (0.614), arginine ($r = 0.507$), histidine ($r = 0.470$) and valine ($r = 0.486$).

Changes in the mean plasma concentrations of amino acids were comparable to our previously published data (Ward et al., 1999) the majority showing significant decreases immediately and 24 h post Marathon, such an adaptation being due primarily to their utilisation for gluconeogenesis. However, in contrast, the mean taurine concentrations were significantly elevated both post race, $P < 0.01$ and after 24 h, $P < 0.05$.

The physiological response by the muscle to exhaustive exercise, particularly with regard to changes in plasma and urinary taurine concentrations remain to be elucidated, but is probably related to muscle function impairment. The increase in taurine urinary excretion could be used as an indicator of muscle damage occurring during exhaustive exercise. Whether taurine supplementation would minimise such changes is an interesting scientific question and merits investigation.

Keywords: Amino acids – Taurine – Exercise – Urine – Plasma

Introduction

The zwitteronic, sulphonated amino acid taurine is present at relatively high concentrations in human muscles; type I skeletal muscle contains 39.2 mmol·kg^{-1} dry weight by comparison to type IIa and IIb muscle fibres where the taurine content is approximately 4 times lower i.e. 9.6 mmol·kg^{-1} dry weight (Harris et al., 1998). The high concentration of taurine would suggest an important role for this sulphonated amino acid in muscle function. This might involve a pharmacological role, millimolar concentrations of taurine applied in vitro increase the skeletal muscle membrane macroscopic conductance to Cl^- and stabilise the sarcolemma (Conte Camerino et al., 1987). On the other hand, intracellular taurine could have a physiological role in modulating the excitatory-contraction coupling mechanism independently of a Cl^- channel effect, probably by controlling calcium availability. This could involve an interaction between taurine and the Ca^{++} transporters present in the sarcoplasmic reticulum (De Luca et al., 1996). In skeletal muscle, taurine in the millimolar range, increases the sarcoplasmic reticulum rate of calcium uptake (Huxtable and Bressler, 1973). Taurine also may play a more central role in cellular muscle function; it is able to compensate for changes in osmolarity by its movement, together with other osmolytes, out of the cell during periods of hypo-osmolarity with the reverse in hyperosmolar conditions (Huxtable, 1992).

After endurance events, such as a marathon (Ward et al., 1999) and 100 km run (Décombaz et al., 1979; Ward et al., 1999) there are significant increases in plasma taurine content, while methionine and cystine, sulphur containing amino acids show no consistent change, and other amino acids are decreased probably due to their utilisation for gluconeogenesis (Décombaz et al., 1979). The source of such taurine remains unknown although the skeletal muscle is the major candidate (Ward et al., 1999). Since taurine, under normal conditions is effectively recycled via the glomerular filtration system in the kidney, it was of interest to know whether such increases in plasma taurine concentrations were either effectively reabsorbed or excreted. Urinary taurine excretion possibly reflects nutritional intake, as well as protein synthesis or catabolism (Timbrell and Waterfield, 1996) plus a variety of metabolic processes such as its release from tissues. Amino acids are absorbed from the glomerular filtrate in the proximal tubule by mechanisms which vary according to whether the amino acids are present as zwitterionic, cationic or anionic species.

Therefore in these present studies, urine specimens were collected from trained athletes competing in a marathon, initially at the start, within 60 minutes of the completion of the marathon and then 24 h after its completion, in order to investigate any changes in taurine excretion as well as other amino acids. In parallel, blood specimens were also collected for the analysis of plasma taurine and amino acids.

Methods

Subjects and sampling procedure

Eight trained athletes, entering the Rotterdam marathon, 1998, agreed to participate in this study. Their mean time for the completion of the marathon was 205.2 ± 18.2 min. Blood and urine specimens were taken from each competitor on three occasions: 30 minutes before the start of the race, within sixty minutes of its completion and twenty four hours later. The urinary debit ($ml \cdot min^{-1}$) was measured by collecting the urine produced during an approximate 3 hour period preceding the start of the event, at the end of the marathon, and during another 3 hour period the day after the race. The duration of each urine collection was noted precisely. During the event, the runners drank freely of the beverage Isostar which did not contain taurine.

Biochemical analyses

EDTA blood specimens were taken for haematological investigations which included haemoglobin, haematocrit, white cell, red cell and platelet counts.

Lithium heparin blood specimens were divided into two, one was centrifuged at 1,500 g for 15 minutes to isolate plasma, while the remaining sample was spun at 300 g for 10 minutes to obtain cell rich plasma.

Proteins were precipitated in the plasma and cell rich plasma samples prior to amino acid analysis by an automated technique (Pharmacia Biotech biochrome 20) with UV detection after reaction with ninhydrin reagent. Total plasma tryptophan content was assayed by fluorimetry by the modified method of Bloxam and Warren (1974) based on the original method of Denckla and Dewey (1967).

Plasma creatine kinase was assayed by standard biochemical techniques. Total DNA in cell rich plasma was quantified by fluorimetric assay after reaction with DAPI (Kapuscinski and Skoczylas, 1977; Brunk et al., 1978).

The volume of urine and the time of collection were noted to calculate urinary flow rate. An aliquot was taken for amino acids analysis and creatinine was assayed by standard biochemical techniques.

The results for the plasma amino acids, haematological parameters and creatine kinase activities were corrected for changes in plasma volume. Changes in plasma volume were calculated by the formula of Dill and Costill (1974):

$$100 \times \left(\frac{\left[Hb\right]_{ref} \times \left(100 - Hct\right)}{\left[Hb\right] \times \left(100 - Hct_{ref}\right)} - 1 \right)$$

[Hb] is the heamoglobin concentration, Hct is the haematocrit expressed in % and corrected for trapped plasma (0.96) and venous vs. whole body Hct (0.93), the reference (*ref*) is the pre-race value.

By using the formulas

$$\text{Average urinary concentration} \times \text{Urinary flow rate}$$

$$\frac{\text{Average urinary concentration} \times \text{Urinary flow rate}}{\text{Blood concentration}}$$

respectively the excretion and the clearance of taurine and each amino acid in the urine were calculated.

Statistical analyses

Comparisons of pre and post marathon results were carried out by paired Student t-tests. Level of significance was set at 0.05, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Correlation significances were assayed by Pearson tests. Data are presented as mean, median and range.

Results

Significant alterations in haematological indices after correction for changes in plasma volume were apparent in each of the subjects both post-race and after the first 24 h of recovery (Table 1). The erythrocyte counts decreased significantly ($P < 0.001$) in the 24 h sample by comparison to the pre-race specimens. Leucocyte counts increased significantly from their pre-race after completing the marathon ($P < 0.001$). The 24 h sample showed significant decreases in platelet numbers by comparison to the pre-race samples ($P < 0.01$) (Table 1).

Figure 1A shows the mean plasma concentrations of taurine and amino acids corrected for plasma volume changes. Plasma taurine content had increased significantly by approximately two fold, after the completion of the marathon ($P = 0.003$). The majority of amino acids diminished significantly in the post-race sample, although the sulphonated amino acid methionine as well as glutamic acid, tyrosine, phenylalanine, isoleucine, cystine and tryptophan showed no significant alteration. Twenty four hours later, the plasma amino acids with the exception of arginine, proline, cystine and glycine had increased towards their pre-race value, while the mean taurine concentration was still significantly elevated ($P = 0.033$).

Analysis of the cellular amino acid content, calculated by the formula (cell rich plasma taurine content – plasma taurine content) per g DNA rich plasma (Fig. 1B) failed to show any differences in the amino acid content in any of the 3 samples collected apart from cystine ($P = 0.016$) which increased in the post-race sample.

Figure 2 shows the urinary excretion rate of taurine and amino acids expressed as $\mu mol \cdot min^{-1}$. Pre-race values for urinary amino acid content showed that the excretion of glycine was high (2.1, 2.3 (0.7–3.0) $\mu mol \cdot min^{-1}$)

Table 1. Haematological parameters corrected for plasma volume changes before, after and 24 h after a marathon run

	Pre-race	Post-race	24 h
Changes in plasma volume (%)	–	5.2 ± 8.3 NS	14.5 ± 6.9 ***
Erythrocytes ($*10^6 \cdot mm^{-3}$)	5.23 ± 0.32	4.93 ± 0.48 NS	4.28 ± 0.54 ***
Leucocytes ($*10^3 \cdot mm^{-3}$)	5.97 ± 1.21	15.37 ± 2.98 ***	6.31 ± 1.84 NS
Platelets ($*10^3 \cdot mm^{-3}$)	264 ± 43	257 ± 59 NS	208 ± 38 **

Data are presented as mean ± SD. Paired Student statistic in comparison to pre-race data is shown. NS $P > 0.05$, ** $P < 0.01$, *** $P < 0.001$.

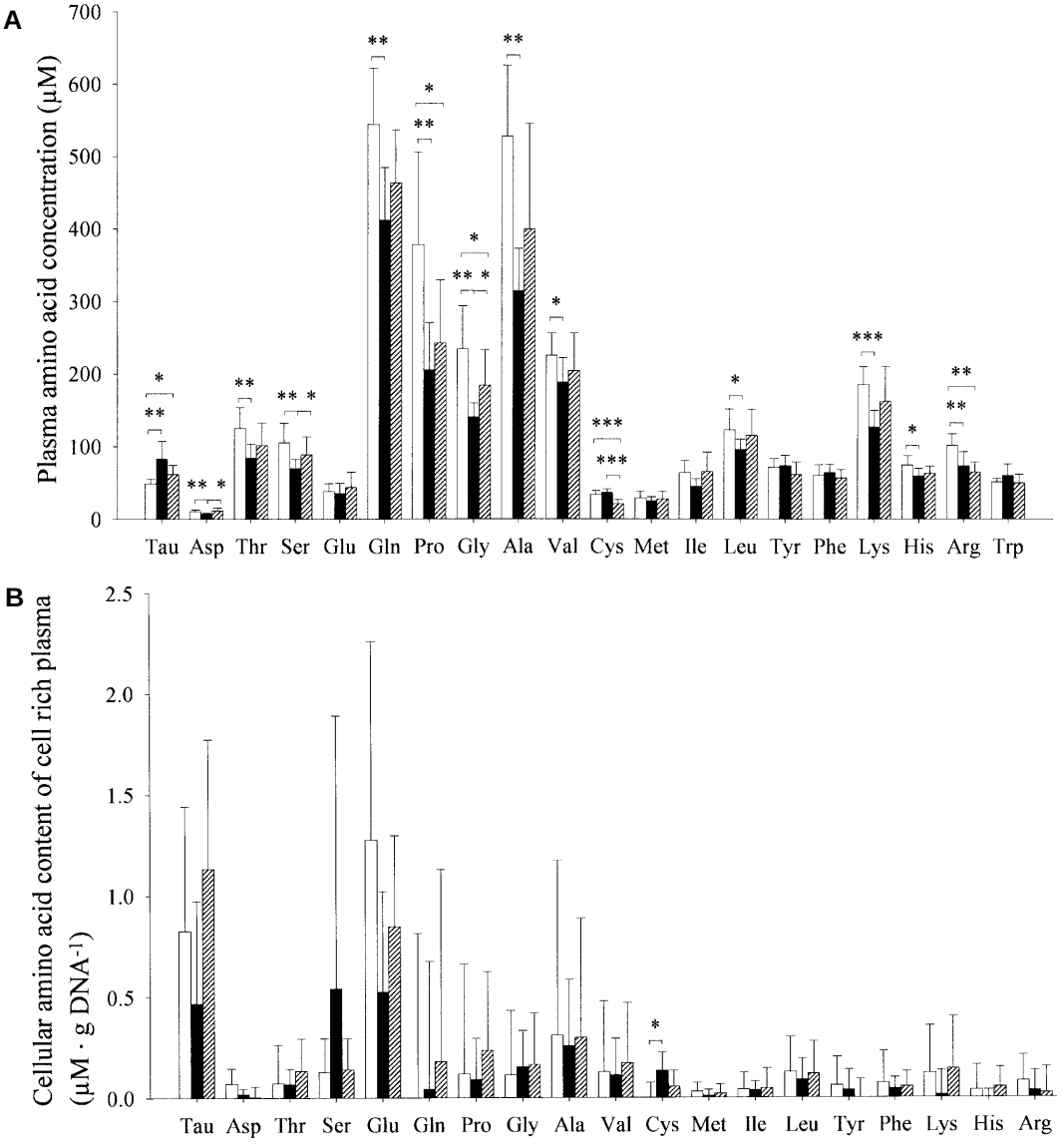

Fig. 1A. Plasma taurine and amino acid concentrations expressed in μM and corrected for changes in plasma volume in comparison to pre-race volume. **B.** Cellular amino acid concentrations of cell rich plasma calculated as the difference between cell rich plasma content and plasma content and divided by the DNA content. Data are presented as mean $\pm$ S.D. of data taken before (white), after the marathon (black), and 24 hours later (hatched). Paired Student t-test significance is shown, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

while all of the other amino acids, as well as taurine, were less than $1\,\mu$mol·min^{-1}. Although there was a three fold increase in mean urinary taurine excretion at the completion of the marathon the range of results was wide, varying from 7% of the original pre-marathon value to in excess of 2,640% which would have contributed to the non-significant changes. In contrast, most of the amino acids decreased in concentration immediately

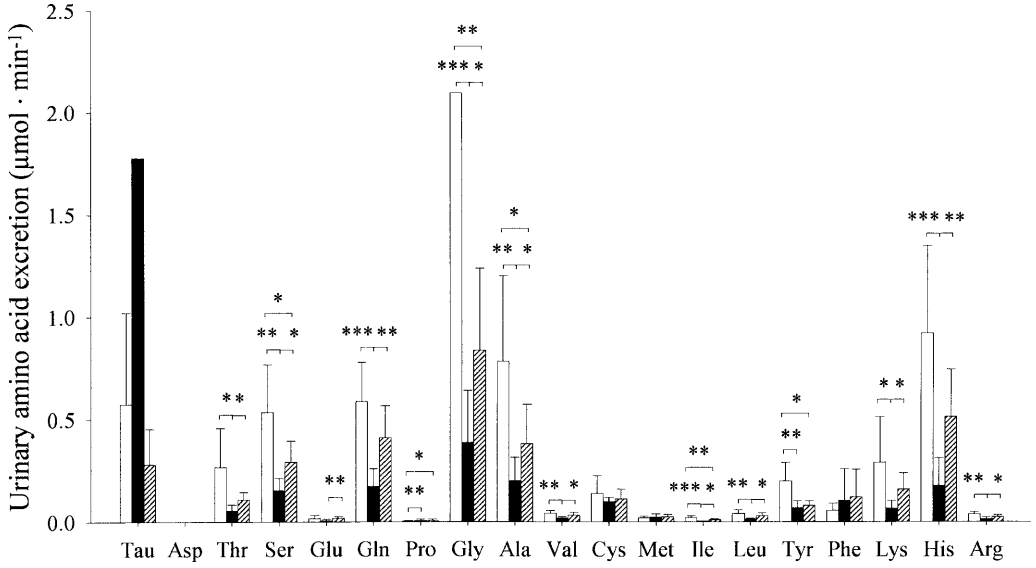

Fig. 2. Urinary taurine and amino acid excretion rate expressed as μmol per min. Data are presented as mean ± S.D. of data taken before (white), after the marathon (black), and 24 hours later (hatched). The S.D. for post-race taurine concentration is 2.06, and for pre-race glycine is 0.82 (not shown). Paired Student t-test significance is shown, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

after the marathon. Twenty four hours post marathon, the mean urinary taurine content had returned to its pre-marathon value. Relationship between the excretion rate of urinary amino acids and the corresponding plasma sample content showed high correlation ($P < 0.001$) between amino acids in these two biological fluids for glycine ($r = 0.853$), threonine ($r = 0.749$), and alanine ($r = 0.747$). Very good correlation ($P < 0.01$) was found for taurine ($r = 0.528$), serine ($r = 0.620$) and glutamine (0.614) and also ($P < 0.05$) for arginine ($r = 0.507$), histidine ($r = 0.470$) and valine ($r = 0.486$). Figure 3 shows the mean clearance of taurine and amino acids at each of the three time points investigated in the marathon runners. On completion of the marathon, the urinary clearance of taurine had not changed significantly (11.7, 11.1 (2.2–26.9) to 18.5, 12.1 (0.7–58.5) ml·min^{-1}), (mean, medium, range), although its mean value had increased two fold. Most of the amino acids showed significant decreases, apart from the sulphonated amino acids methionine and cystine and also lysine, glutamic acid and phenylalanine where no significant alterations in their clearance had occurred immediately post marathon. Twenty four hours after the marathon, urinary clearance of taurine was significantly reduced ($P = 0.041$) by comparison to the pre-race value. Some of the amino acids still showed a significant decrease by comparison to the pre-race value, i.e. threonine, glycine, alanine, isoleucine and tyrosine. Proline clearance showed a significant increase, all other amino acids were now similar to their pre-race value.

Table 2 shows the mean values of plasma creatine kinase activity corrected for plasma volume changes. A significant increase in its mean concen-

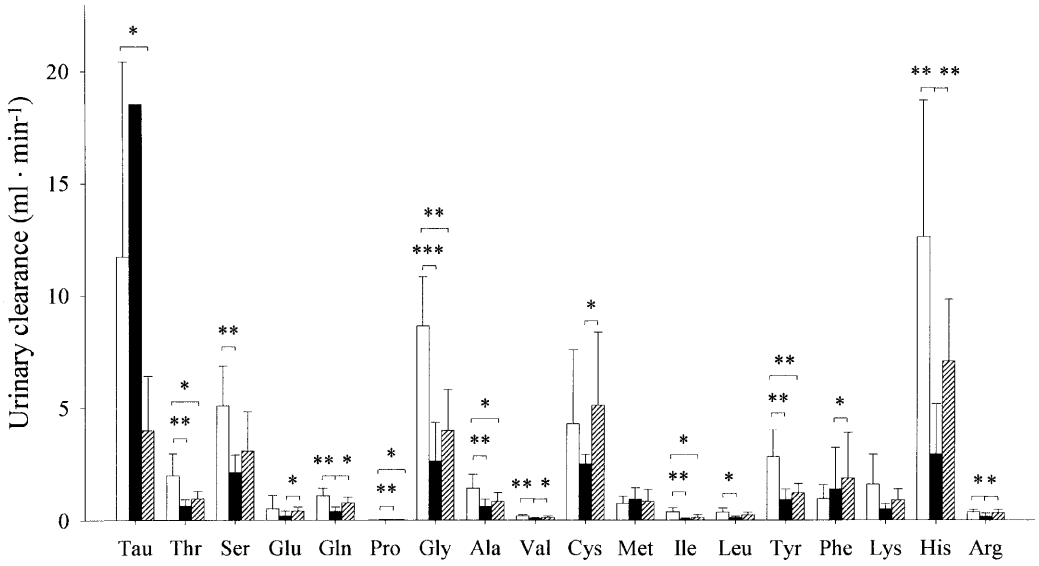

Fig. 3. Urinary clearance of taurine and amino acids. Data are presented as mean ± S.D. of data taken before (white), after the marathon (black), and 24 hours later (hatched). The S.D. for post race taurine concentration is 20 (not shown). Paired Student t-test significance is shown, *P < 0.05, **P < 0.01, ***P < 0.001

Table 2. Plasma creatine kinase and plasma, urinary and cellular taurine content before, after and 24 h after a marathon run

	Pre-race	Post-race	24 h
Creatine kinase ($U \cdot l^{-1}$)	106 ± 33	354 ± 297 *	993 ± 1315 NS
Plasma taurine (μM)	48.9 ± 6.6	82.7 ± 24.7 **	61.8 ± 11.4 *
Urinary taurine excretion ($\mu mol \cdot min^{-1}$)	0.57 ± 0.45	1.78 ± 2.06 NS	0.28 ± 0.17 NS
Cellular taurine of cell rich plasma ($\mu M \cdot g\ DNA^{-1}$)	0.83 ± 0.62	0.47 ± 0.51 NS	1.13 ± 0.64 NS

Plasma creatine kinase and plasma taurine are corrected for changes in plasma volume. Cellular taurine content of cell rich plasma is calculated as the (cell rich taurine content – plasma taurine content) per g DNA rich plasma. Data are presented as mean ± SD. The Paired Student statistic in comparison to pre-race data is shown. NS P > 0.05, * P < 0.05, ** P < 0.01.

tration was observed in the sample taken immediately after the completion of the marathon (P = 0.04). Furthermore, when the individual results for the percentage change in urinary taurine excretion rate between pre and post-race value were correlated with creatine kinase activity increases between pre and post-race values (r = 0.972, P < 0.001) or between pre and 24 h later values (r = 0.872, P < 0.001), positive correlations were observed (Fig. 4). The increases in creatine kinase activities did not correlate with changes in either plasma or cellular taurine content between pre and post race data.

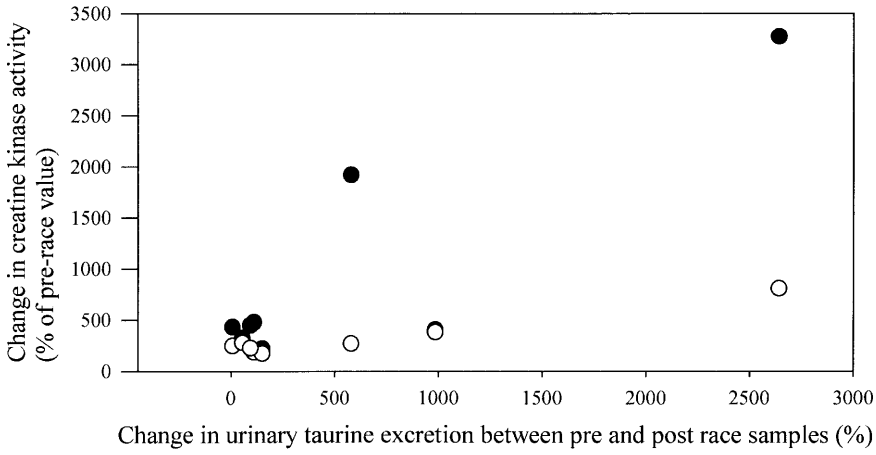

Fig. 4. Individual percentage change between pre and post race samples in urinary taurine excretion level, expressed in μmol per min, which is correlated with percentage change in blood creatine kinase level, corrected for plasma volume changes, between pre and post race samples (in white, $r = 0.972$, $P < 0.001$), and between pre and 24 h post race sample (in black, $r = 0.872$, $P < 0.001$)

Discussion

After endurance events, such as the marathon or 100 km run, there are extensive changes in a variety of plasma amino acids which are utilised as substrate for gluconeogenesis. (Poortmans, 1993), e.g. alanine transfer from muscle to blood will increase with the intensity of the exercise, any excess being taken up by the liver and converted back to glucose to ultimately be utilised by muscle. In the present study, the majority of other amino acids which can be utilised for gluconeogenesis, namely threonine, serine, glutamine, proline, glycine, alanine, valine, histidine and asparagine all showed significant decreases at the completion of the marathon. However, the plasma taurine content is the only amino acid which increased significantly at the completion of the marathon. Other sulphur containing amino acids, such as cystine and methionine showed no significant alterations at the end of the marathon. Twenty four hours later, the majority of amino acids had reached their initial basal concentration, only proline, arginine, cystine and glycine were still reduced, while taurine remained significantly elevated.

High levels of creatine kinase and other muscle proteins are detectable within the plasma for several days after marathon running (Young, 1984). Furthermore it is claimed that the greatest increases will be found in the slowest runners, suggesting greater skeletal muscle damage in the least trained or fit (Haibach and Hosler, 1985). What was noteworthy in this present study was that there was a very strong correlation with the percentage change in the urinary taurine excretion in the pre and post race samples when high creatine kinase activities were assayed in the plasma at the completion of the marathon ($r = 0.972$, $P < 0.001$), and after 24 h ($r = 0.872$, $P < 0.001$). Such results may indicate that in such subjects there was excessive muscular dam-

age which resulted in the release of both creatine kinase and taurine, such taurine then being excreted in the urine. In contrast, in the other subjects in the marathon race where creatine kinase values did not show such an increase, little change in urinary taurine was found. Therefore, the increase in taurine urinary excretion could be used as an indicator of muscle damage occurring during exercise. Nevertheless, it remains unclear as to why the muscle is unable to reacquire such taurine which is released but could reflect changes in the ability of the taurine transporters which facilitate taurine uptake by the muscles. In contrast, Iwata et al. (1986) showed that muscle stimulation increased the rate of transport of taurine into the muscle. However, concomitant release of taurine from the muscle in such a situation has never been investigated.

In our previous studies (Ward et al., 1999), we had been unable to identify the source of the elevated plasma taurine content and concluded that it might be released from the muscle or perhaps from excessive leucocyte and platelet lysis, both of these cell types being rich in taurine (Huxtable, 1992). Despite the fact that there was an increase in leucocytes in the blood immediately after the completion of the marathon, we were unable to detect any change in the cellular taurine content of the cell rich plasma. We must therefore conclude that such changes in leucocyte cells do not significantly contribute to increased circulating concentrations of taurine.

These studies have confirmed some of the earlier studies of changes in a number of amino acids in the plasma after the completion of a marathon (Conlay et al., 1989; Blomstrand and Newshome, 1992; Bazzarre et al., 1992). Such studies have been extended to investigate whether informative changes also occur in the urine, a biological fluid which could be used in preference to the invasive approach of taking blood specimens.

In this present study, no consistant alteration in the urinary taurine excretion was assayed at the conclusion of the marathon, six subjects showing an increase in taurine, although the variation of results between the subjects was wide. In the study of Decombaz et al. (1979), which investigated 100 km runners, similarly no significant alteration in the excretion rate of taurine was evident. Clearly there are a combination of factors including glomerular filtration changes as well as blood osmolarity, which might influence taurine release from cells, which may influence urinary taurine excretion. In contrast, most of the urinary amino acids showed a net decrease in excretion which clearly reflected the decrease in plasma content as well as the efficiancy of the kidney to effectively recycle them. A reduction in renal blood flow occurs during endurance events, which is paralleled by an alteration in the concentrating ability of the kidney (Refsum and Stromme, 1975). It is reported (Decombaz et al., 1979) that amino acids with a renal clearance at rest under $1\,ml \cdot min^{-1}$ (e.g. isoleucine, arginine, valine, methionine, leucine and lysine) retain a higher relative clearance during an endurance event, in contrast to amino acids with a resting clearance above $1\,ml \cdot min^{-1}$ (e.g. alanine, glutamic acid, threonine, phenylalanine, cystine, tyrosine, asparagine, glycine, serine, taurine, aspartate and histidine) which decrease. However this was not confirmed in this present study. Other endurance events, e.g. physical load on

a bicycle ergometer, also showed significant decreases in both urinary excretion and clearance of amino acids (Vlcek and Stemberk, 1990) to that reported in this present study.

Changes in the urinary amino acid excretion of all amino acids except aspartate, glutamic acid, proline, cystine, methionine, isoleucine, leucine, tyrosine, phenylalanine and lysine paralleled the changes in plasma parameters. Since there are certain problems associated with the collection of blood specimens which include the limited blood circulation in the periphery after exhaustive exercise (most of the circulation being in the cardiovascular system), the separation and storage of the plasma samples as well as the necessity for medical personnel for the collection of the blood specimens, an alternative biological fluid which could be collected by a non-invasive technique, which showed comparable changes to that of plasma could be of great use. Clearly the collection of an individual urine sample at certain intervals before and after the endurance event would be of relatively ease, particularly if it can be shown that some meaningful interpretation of the urinary amino acids would reflect changes in the blood circulation.

Although overall the changes in urinary taurine were not informative, it was of particular note that where there was a high percentage change in urinary taurine excretion levels there was a strong correlation with an increased plasma creatine kinase activity, thus supporting our earlier study (Ward et al., 1999) that increases in taurine content of either plasma or urine are related to loss from skeletal muscle. Furthers studies with larger numbers of subjects competing in endurance events are clearly warranted to confirm these observations.

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