Amino Acids (1998) 15:241–251

Amino
Acids

Effect of regular training on the myocardial and plasma concentrations of taurine and α-amino acids in thoroughbred horses

N. King and **M.-S. Suleiman**

Bristol Heart Institute, University Departments of Cardiac Surgery and Physiology, Bristol Royal Infirmary, Bristol, United Kingdom

Accepted January 21, 1998

Summary. Exercise induces significant changes in the free intracellular amino acid pool in skeletal muscle but little is known of whether such changes also occur in cardiac muscle. In this study the effect of regular exercise on the size and the constituents of the free amino acid pool in the hearts and in the plasma of thoroughbred horses was investigated. The total free intracellular amino acid pool in the hearts of control horses was 30.9 ± 1.2 μmol/g wet weight (n = 6). Glutamine but not taurine was present at the highest concentration (13.5 ± 0.9 and 7.7 ± 0.69 μmol/g wet weight for glutamine and taurine respectively). As for the rest of the amino acids in the pool, only glutamate and alanine were present at levels greater than 1 μmol/g wet weight (4.6 ± 0.25 and 1.7 ± 0.14 for glutamate and alanine respectively). The tissue to plasma ratio was highest for taurine at 155, followed by glutamate at 111, aspartate and glutamine at 37, alanine at 5.8 and ratios of less than 3 for the rest of the amino acids. The total free intracellular amino acid pool in the hearts of exercised horses was slightly but not significantly lower than control (28.1 ± 1.1 μmol/g wet weight, n = 6). Regular exercise increased the intracellular concentration of threonine, valine, isoleucine, leucine and phenylalanine but was only significant ($p < 0.05$) for threonine.
This work has documented the profile of taurine and protein amino acids in the heart and in the plasma of thoroughbred horses and showed that in contrast to skeletal muscle, heart muscle does not show major changes in amino acids during regular exercise.

Keywords: Amino acids – Taurine – Protein amino acids – Thoroughbred horses – Heart – Plasma

Introduction

Regular training or intensive exercise are known to induce changes in the intracellular concentration of free amino acids in skeletal muscle (MacLean et

al., 1994; van Hall et al., 1995; Graham et al., 1995). For example trained men have higher muscular concentrations of taurine, alanine, glutamate and phenyalanine but have lower plasma glutamate concentration than untrained subjects (Graham et al., 1995). These changes could be in part due to effects of exercise on amino acid transport mechanisms in skeletal muscle (King, 1994).

In contrast to skeletal muscle little is known of whether training or exercise influences the free intracellular amino acid pool in heart cells. This pool, which is largely made up of the non-protein β-amino acid taurine and the non-essential amino acids, is quite large with concentrations in excess of 30 μmol/g wet weight for most mammalian hearts (Huxtable et al., 1981; Suleiman et al., 1993; Suleiman and Chapman 1993; Suleiman et al., unpublished data). The size and the constituents of the pool are different for different mammalian hearts. Furthermore, several of the amino acids comprising this pool are important for normal cellular function. For example taurine deficiency is associated with development of cardiomyopathy, possibly by influencing cellular calcium homeostasis and membrane stabilisation (Huxtable 1992; Punna et al., 1994; Schaffer et al., 1994; Suleiman, 1994). Although the significance of the non-essential amino acid pool has been largely viewed in terms of protein breakdown and synthesis, individual amino acids contribute to a variety of other cellular activities. Glutamate metabolism in the heart is particularly prominent (Rosenkranz, 1995; deVillalobos and Taegtmeyer, 1995). It is involved in major transamination reactions and its keto acid is an important participant within the Krebs cycle. Glutamate and aspartate are important for the malate-aspartate shuttle by which cytoplasmic reducing equivalents, generated by glycolysis, are transferred into the mitochondria for energy production. Glutamine is a nitrogen donor for the biosynthesis of a number of important compounds such as nucleotides and amino acids (see Rennie et al., 1994).

It is evident therefore that a change in the free intracellular amino acids in the heart during exercise, may have important implications for myocardial function. In this study we have documented the content of the intracellular amino acids pool in horse's heart and in the plasma and have monitored the effect of regular training on these amino acids.

Materials and methods

Collection of hearts

Hearts were obtained from twelve thoroughbred horses, six of which had been subjects in a short term regular exercise programme (16 weeks), whilst the other six were controls. At the completion of the study the mean age and body mass of the control group was 23.6 $\pm$ 1.2 months and 413.1 $\pm$ 6.12 kg ($n = 6$, $\pm$ SEM) respectively, compared to 23.6 $\pm$ 1.2 months and 395 $\pm$ 5.45 kg for the exercised group. There were no significant differences ($p < 0.05$) between these values, neither was there any significant gain or loss of weight during the course of the study in either group.

Blood samples were collected prior to sacrificing the animals. Euthanasia was achieved by pre-treatment with an injection of "Domosedan" (Norden Laboratories)

containing Detomidine hydrochloride (10 mg/ml), followed by an overdose of "Somulose" (Arnolds) containing Quinalbarbitone sodium (400 mg/ml) and Cinchocaine hydrochloride (25 mg/ml). The throat was then slit, the chest cavity opened, the heart removed, weighed and submerged in an excess of cold cardioplegic solution containing (in mM) 147NaCl, 20KCl, 16$MgCl_2 \cdot 6H_2O$, 2$CaCl_2$ and 1 tetracaine HCl (pH 7.4 with NaOH). There was no significant difference in the weights of the hearts between the two groups: 3.05 ± 0.06 kg for control compared to 3.21 ± 0.05 kg for regularly exercised animals. Ventricular myocardial biopsy specimens were immediately collected and frozen in liquid nitrogen until processing for amino acid analysis.

Determination of amino acids in myocardial tissue

Amino acids were extracted using an extraction procedure described previously (Suleiman et al., 1997a,b). In brief, frozen tissues were crushed under liquid nitrogen and the resultant powder was extracted with perchloric acid. The extracts were centrifuged at 1500 g for 10 min at 4°C. The protein containing pellet was resuspended and protein was determined according to the Lowry method (Lowry et al., 1951) using a kit from Sigma (Poole, Dorset, UK), with bovine serum albumin (Sigma, UK) as a standard. The supernatant was neutralised and amino acids were determined according to the Waters Pico-Tag method (Cohen et al., 1989; Suleiman et al., 1997a,b). Essentially, 100 μl of the extract was dried using vacuum centrifugation and free amino acids were derivatized using phenylisothiocyanate. The phenylisothiocarbamyl derivatized amino acids were separated by HPLC using a 30 cm Pico-Tag column (Millipore Corporation, Milford, MA, USA) with two Waters delivery pumps (A & B) at a constant flow of 1 ml/min with the following gradient: 100% A for 13.5 min, 97% A for 10.5 min, 94% A for 6 min, 91% A for 20 min, 66% A for 12.5 min and 0% A for 4 min. The solvents used were for A: 132 mM Na Acetate, 470 ml/l triethylamine, pH 6.4 and 6% acetonitrile. Solvent B was 60% acetonitrile. Derivatized amino acids were detected at 254 nm (46°C) using a Waters 486 detector. Quantitative and qualitative analysis was carried out using amino acid standards (Sigma, Dorset UK) and the acquired data was processed using the Millenium 2000 software supplied by Waters, Millipore (UK) Ltd., Watford, Herts.

Chemicals needed to derivatize amino acids and separate them were obtained from Waters, Millipore (UK) Ltd., Watford, Herts.

Intracellular amino acids concentrations were expressed per wet weight and per protein content.

Amino acid determination in plasma

Plasma was deproteinised using Millipore 5K molecular weight cut-off limit ultrafiltration units for 15 min at 8500 g. The procedure for amino acid determination was the same as described above.

Data collection and analysis

The mean values of the amino acid levels in myocardial tissue and in the plasma were calculated for each horse. Measurements for each horse were carried out in duplicate and the difference between the two measurements did not exceed 5%. The mean values for each horse were then used to calculate the mean value (± standard error) for each group. Intergroup differences were analysed by ANOVA (Fisher's PLSD) using a statview package provided on a Macintosh PC. The level of statistical significance was taken as 95%.

The correlations between pairs of continous variables were computed using the "listwise deletion" method available on the statview package. Both the correlation matrix and p-values (using the statistical test Fisher's r to z) were obtained. The correlation matrix value is a measure of the strength of a linear relation between two variables ranging between −1(strong negative correlation) to +1(strong positive correlation). A correlation close to zero will indicate that there is no relation between the corresponding variables. The significance of the correlation matrix is that it enables the prediction of one variable if the other is known, allows for a simplified description of the data and the relation can be used to hypothesise mechanisms which cause the variables to be related.

Results

Table 1 shows the intracellular concentration of the total free intracellular amino acid pool in hearts of control or regularly exercised thoroughbred horses. The size of this pool was slightly, though not significantly lower in horses that have undergone regular exercise. When the non-protein amino acid taurine was excluded, the trend remained although the difference between the two groups was further reduced. Taurine at a concentration of 7.7 ± 0.69 μmol/g wet weight constitutes 24% of the total free intracellular amino acid pool (Table 2). On the other hand, the concentration in the plasma was more than a 100 fold lower giving rise to a large transmembrane concentration gradient.

Table 3 shows the effect of regular exercise on the total content of three groups of amino acids: essential, non-essential or branched-chain amino acids. Only the branched-chain amino acids showed a clear trend to increase in the hearts of exercised horses.

Table 4 shows the intracellular concentration of the non-essential amino acids. These amino acids along with taurine constitute more than 90% of the free intracellular amino acid pool. Glutamine at 13.5 μmol/g wet weight is by far the largest contributor to the pool (constitutes 43% of the pool). Apart from taurine, glutamate (15%) and alanine (5.7%), the rest of the amino acids

Table 1. The intracellular concentration of the free amino acid pool (with or without taurine) in the hearts of thoroughbred horses with or without regular exercise. The concentration is expressed per wet weight and per protein content. Values are mean ± SEM (n = 6). Level of significance refers to exercise versus corresponding control

Concentration units		Control	Regular exercise	Levels of significance (p values)
μmol/g wet weight				
	Total amino acid pool			
	With taurine	30.9 ± 1.2	28.1 ± 1.1	0.11
	Without taurine	23.2 ± 1.2	21.6 ± 1.5	0.4
μmol/g protein				
	Total amino acid pool			
	With taurine	277 ± 17	257 ± 8	0.3
	Without taurine	209 ± 15	197 ± 12	0.56

Table 2. The concentration of taurine in the hearts and in the plasma of thoroughbred horses with or without regular exercise. Also shown are the percentage of taurine in the pool and the tissue/plasma ratios. Values are mean ± SEM (n = 6 for each group). Level of significance refers to exercise versus corresponding control

	Control	Regular exercise	Level of significance (p values)
Tissue concentration (μmol/g wet weight)	7.7 ± 0.69	6.5 ± 0.84	0.32
(% of total)	(24.8 ± 2.2)	(23.5 ± 3.6)	
Tissue concentration (μmol/g protein)	68.3 ± 6.4	59.9 ± 8.8	0.46
(% of total)	(24.8 ± 2.2)	(23.4 ± 3.6)	
Plasma concentration (μM)	146 ± 76	100 ± 66	0.65
Tissue/plasma ratio (Tissue wet weight concentration/plasma concentration)	155 ± 47	173 ± 42	0.76

Table 3. Changes in the non-essential, essential and branched-chain amino acids in the hearts of thoroughbred horses with or without regular exercise. Values are mean ± SEM (n = 6 for each group). Level of significance refers to exercise versus corresponding control

	Control	Regular exercise	Level of significance (p values)
Non-essential amino acids (μmol/g wet weight)	21.78 ± 1.1	20.02 ± 1.5	0.35
Non-essential amino acids (μmol/g protein)	195.9 ± 14.6	182.9 ± 11.9	0.50
Essential amino acids (μmol/g wet weight)	1.47 ± 0.2	1.53 ± 0.08	0.78
Essential amino acids (μmol/g protein)	13.1 ± 1.7	14.16 ± 1.2	0.62
Branched chain amino acids (μmol/g wet weight)	0.26 ± 0.02	0.30 ± 0.02	0.14
Branched chain amino acids (μmol/g protein)	2.3 ± 0.2	2.79 ± 0.2	0.10

were present at less than 5% each. There was no significant effect of regular exercise on the non-essential amino acids, although a trend to decrease in glutamate and proline and a trend to increase in serine and asparagine was seen in the heart of exercised horses.

In contrast to the non-essential amino acids, essential amino acids showed clear trends in their levels as a result of regular exercise. An increase in the intracellular concentration of threonine, valine, isoleucine, leucine and phenylalanine was seen in the heart of exercised horses (Table 5). This increase was statistically significant ($p < 0.05$) for threonine only.

It is interesting to note that the increase in tissue threonine was also associated with elevated plasma levels of the amino acid in the exercised

Table 4. The intracellular concentration of the non-essential amino acids in the hearts of thoroughbred horses with or without regular exercise. Also shown (in parenthesis) the percentage of individual amino acids in the total pool (including taurine). The concentration is expressed per wet weight and per protein content. Values are mean ± SEM (n = 6 for each group). Level of significance refers to exercise versus corresponding control

Amino acid	Tissue concentration (μmol/g wet weight) (% of total)			Tissue concentration (μmol/g protein) (% of total)		
	Control	Regular exercise	p values	Control	Regular exercise	p values
Aspartate	0.34 ± 0.04 (1.1 ± 0.1)	0.36 ± 0.03 (1.3 ± 0.12)	0.59	3.38 ± 0.54 (1.2 ± 0.13)	3.56 ± 0.47 (1.4 ± 0.16)	0.4
Glutamate	4.6 ± 0.25 (14.9 ± 0.63)	4.2 ± 0.3 (15.0 ± 1.4)	0.34	41.2 ± 3.2 (14.9 ± 0.6)	38.6 ± 4 (15 ± 1.4)	0.63
Asparagine	0.06 ± 0.01 (0.19 ± 0.02)	0.07 ± 0.01 (0.26 ± 0.04)	0.28	0.51 ± 0.04 (0.19 ± 0.02)	0.67 ± 0.11 (0.26 ± 0.04)	0.20
Serine	(0.4 ± 0.02 (1.3 ± 0.06)	0.5 ± 0.05 (1.8 ± 0.19)	0.12	3.6 ± 0.26 (1.3 ± 0.06)	4.6 ± 0.6 (1.77 ± 0.19)	0.14
Glutamine	13.5 ± 0.9 (43.7 ± 2.1)	12.3 ± 1.2 (43.5 ± 2.9)	0.44	121.5 ± 10 (43.6 ± 2)	111.7 ± 8.8 (43.4 ± 2.9)	0.48
Glycine	0.93 ± 0.15 (2.9 ± 0.39)	0.82 ± 0.1 (2.9 ± 0.27)	0.59	8.4 ± 1.6 (2.9 ± 0.4)	7.6 ± 0.9 (2.9 ± 0.27)	0.67
Alanine	1.7 ± 0.14 (5.7 ± 0.6)	1.6 ± 0.096 (5.7 ± 0.17)	0.48	15.4 ± 1.3 (5.7 ± 0.6)	14.6 ± 0.69 (5.7 ± 0.16)	0.62
Proline	0.2 ± 0.02 (0.66 ± 0.08)	0.16 ± 0.025 (0.56 ± 0.07)	0.26	1.96 ± 0.3 (0.72 ± 0.11)	1.58 ± 0.08 (0.62 ± 0.04)	0.24

Table 5. The intracellular concentration of the essential amino acids in the hearts of thoroughbred horses with or without regular exercise. The percentage of individual amino acids in the pool (including taurine) is shown in parenthesis. The concentration is expressed per wet weight and per protein content. Values are mean ± SEM (n = 6 for each group). Level of significance refers to exercise versus corresponding control

Amino acid	Tissue concentration (μmol/g wet weight) (% of total)			Tissue concentration (μmol/g protein) (% of total)		
	Control	Regular exercise	p values	Control	Regular exercise	p values
Histidine	0.26 ± 0.013 (0.85 ± 0.05)	0.25 ± 0.016 (0.89 ± 0.03)	0.62	2.32 ± 0.09 (0.85 ± 0.05)	2.29 ± 0.11 (0.89 ± 0.03)	0.80
Arginine	0.5 ± 0.1 (1.6 ± 0.3)	0.5 ± 0.1 (1.8 ± 0.41)	0.97	4.52 ± 0.98 (1.6 ± 0.3)	4.74 ±1.1 (1.82 ± 0.4)	0.89
Threonine	0.11 ± 0.01 (0.35 ± 0.03)	0.16 ± 0.01 (0.55 ± 0.03)	0.02	0.95 ± 0.1 (0.34 ± 0.03)	1.4 ± 0.06 (0.55 ± 0.03)	0.003
Valine	0.12 ± 0.01 (0.38 ± 0.02)	0.14 ± 0.01 (0.5 ± 0.04)	0.1	1.04 ± 0.09 (0.38 ± 0.03)	1.3 ± 0.1 (0.51 ± 0.04)	0.066
Isoleucine	0.035 ± 0.003 (0.11 ± 0.007)	0.04 ± 0.004 (0.15 ± 0.01)	0.18	0.31 ± 0.03 (0.11 ± 0.01)	0.39 ± 0.04 (0.15 ± 0.02)	0.14
Leucine	0.11 ± 0.01 (0.34 ± 0.02)	0.12 ± 0.005 (0.42 ± 0.03)	0.3	0.94 ± 0.08 (0.34 ± 0.02)	1.09 ± 0.07 (0.42 ± 0.03)	0.22
Phenylalanine	0.043 ± 0.02 (0.14 ± 0.05)	0.073 ± 0.02 (0.26 ± 0.07)	0.27	0.38 ± 0.13 (0.14 ± 0.05)	0.62 ± 0.156 (0.25 ± 0.06)	0.25
Lysine	0.3 ± 0.06 (0.94 ± 0.16)	0.25 ± 0.08 (0.87 ± 0.25)	0.66	2.6 ± 0.5 (0.94 ± 0.16)	2.3 ± 0.76 (0.87 ± 0.24)	0.73

Table 6. The concentration of the essential and non-essential amino acids (μmol/g wet weight) in the hearts and in the plasma (μM) of thoroughbred horses with or without regular exercise. Values are mean ± SEM (n = 6 for each group)

Amino acid	Control			Regular exercise		
	Tissue (μmol/g wet weight)	Plasma (μM)	Tissue/plasma ratio	Tissue (μmol/g wet weight)	Plasma (μM)	Tissue/plasma ratio
Aspartate	0.34 ± 0.04	11 ± 2	37 ± 6	0.36 ± 0.03	8.6 ± 2	61 ± 16
Glutamate	4.6 ± 0.25	42 ± 3	111 ± 8	4.2 ± 0.3	50 ± 10	100 ± 16
Asparagine	0.06 ± 0.01	37 ± 3	1.6 ± 0.2	0.07 ± 0.01	34 ± 4	2 ± 0.2
Serine	0.4 ± 0.02	215 ± 17	1.9 ± 0.08	0.5 ± 0.05	220 ± 15	2.3 ± 0.2
Glutamine	13.5 ± 0.9	367 ± 19	37 ± 1.7	12.3 ± 1.2	364 ± 20	34 ± 3.6
Glycine	0.93 ± 0.15	324 ± 29	2.9 ± 0.5	0.82 ± 0.1	336 ± 21	2.5 ± 0.3
Alanine	1.7 ± 0.14	305 ± 23	5.8 ± 0.6	1.6 ± 0.096	273 ± 27	6.1 ± 0.7
Proline	0.2 ± 0.02	139 ± 15	1.5 ± 0.1	0.16 ± 0.025	106 ± 14	1.6 ± 0.2
Histidine	0.26 ± 0.013	87 ± 5	3.0 ± 0.2	0.25 ± 0.016	93 ± 8	2.8 ± 0.3
Arginine	0.5 ± 0.1	185 ± 15	2.6 ± 0.5	0.5 ± 0.1	176 ± 10	2.8 ± 0.6
Threonine	0.11 ± 0.01	76 ± 7	1.4 ± 0.1	0.16 ± 0.01	99 ± 11	1.6 ± 0.2
Valine	0.12 ± 0.01	165 ± 13	0.7 ± 0.04	0.14 ± 0.01	174 ± 11	0.8 ± 0.08
Isoleucine	0.035 ± 0.003	55 ± 5	0.65 ± 0.04	0.04 ± 0.004	55 ± 3	0.8 ± 0.07
Leucine	0.11 ± 0.01	97 ± 12	1.1 ± 0.05	0.12 ± 0.005	101 ± 7	1.2 ± 0.06
Phenylalanine	0.043 ± 0.02	64 ± 4	0.7 ± 0.2	0.073 ± 0.02	64 ± 3	1.1 ± 0.3
Lysine	0.3 ± 0.06	105 ± 5.4	2.8 ± 0.5	0.25 ± 0.08	95 ± 6	2.6 ± 0.8

group ($p = 0.1$; Table 6). With the exception of threonine, there were no differences between the plasma amino acids levels in control and in exercised horses. The following amino acids were present at concentrations of less than 0.1 mM: Aspartate, glutamate, asparagine, histidine, threonine, isoleucine and phenylalanine. Glutamine, glycine and alanine were present at the highest concentration (>0.3 mM). A calculation of the concentration gradient for amino acids (tissue/plasma) in the control group shows that glutamate has a ratio of 111, followed by aspartate and glutamine at 37 and alanine at approximately 6. The rest of the amino acids had tissue to plasma ratios ranging between 1–3. Similar ratios were also seen for the exercised group (Table 6).

To determine whether changes in the intracellular concentration of amino acids in individual hearts followed a similar pattern, the correlation matrix was calculated (data not shown). A significant positive correlation was seen between aspartate and glutamate, glycine and arginine. There was also a significant positive correlation for glutamate with arginine and for glutamine with histidine and for glycine with lysine. On the other hand the correlation between changes of amino acids in the plasma of individual horses was more widespread (data not shown). For example there was a significant positive correlation between aspartate and both arginine and isoleucine and between glutamate and both leucine and phenylalanine. Also between asparagine with the following: serine, glutamine, histidine, taurine, alanine and valine.

In heart muscle cells, the glutamate keto acid can be used for energy production, especially under ischaemic stress. The keto acid is produced along with alanine, a reaction catalysed by alanine aminotransferase. Subsequently the alanine/glutamate ratio has been used as an index to assess the extent of metabolic stress (Suleiman et al., 1993; Suleiman et al., 1997b). Calculation of this ratio shows no difference between the control (0.38 ± 0.04) and the exercised group (0.39 ± 0.04).

Discussion

This work documents the levels of intracellular amino acid pool in the hearts and in the plasma of thoroughbred horses. The size of the intracellular amino acid pool is similar to those found in guinea-pig (Suleiman and Chapman, 1993), man (Suleiman et al., 1993) and ferret (Suleiman et al., unpublished data). It is smaller than the pool found in rat and mice which is largely due to the high concentration of taurine (Huxtable et al., 1981). However there are differences in the contribution of individual amino acids to the size of the pool in the horse heart when compared to other mammalian hearts. For example unlike other mammalian hearts (including the rat) the β-amino acid taurine is not present at the highest concentration. Instead glutamine constitutes 43% of the pool (compared to 24% for taurine) and therefore is present at the highest concentration. The significance of having such a high concentration of glutamine is not readily apparent. The high concentration of tissue glutamine is not associated with a high level of the amino acid in the plasma. The plasma

concentration of glutamine at 0.37 mM is similar to what is found in other mammalian species. These values provide a tissue/plasma ratio of 37 which is higher than for other mammalian hearts suggesting that hearts of thoroughbred horses tend to accumulate more glutamine. Similar to other mammalian hearts, aspartate, glutamate, glutamine and taurine have high concentration gradients ranging between 37–150. The rest of the amino acids have gradients of less than 5.

Regular exercise of the horses showed a small insignificant change in the size of the amino acid pool. This effect appears to be largely due to a trend to fall in amino acids present at the highest concentration: glutamine, glutamate and taurine. This trend is in contrast to skeletal muscle where training was associated with increased levels in taurine, alanine and glutamate. However like skeletal muscle there was a rise in phenylalanine. Furthermore a strong trend to rise in branched-chain amino acids was also seen. A statistically significant rise was only seen for threonine which was associated with higher plasma levels. The implications of this rise are not readily apparent, although recent work has shown that adding threonine to rats drinking water, elevated systolic blood pressure (Vasdev et al., 1995).

In conclusion this work documents the concentration of the free amino acids in the heart and in the plasma of thoroughbred horses. In addition, the effect of regular exercise on these amino acids was also studied. Although there were no major changes as a result of exercise, there were subtle differences in the intracellular amino acid pool. As these conclusions are based on a small data set, an expanded study with larger sample may be required to further confirm the reported differences. It must be noted however, that such studies are both expensive and rare.

Acknowledgement

We would like to acknowledge Mrs V. Buswell, Mrs D. Wallace and Ms A. Moffatt for their excellent technical assistance. We also would like to acknowledge Prof. A. E. Goodship and Dr. H. L. Birch, Department of Anatomy for organising the study and for providing the hearts. This work was supported by the British heart Foundation and the Garfield Weston Trust.

References

Cohen SA, Meys M, Tarvin TL (1989) Pico-tag advanced methods manual. Millipore Corporation, Bedford, MA, USA

de Villalobos DH, Taegtmeyer H (1995) Metabolic support for postischaemic heart. Lancet 345: 1552–1555

Graham TE, Turcotte LP, Kiens B, Richter EA (1995) Training and muscle ammonia and amino acid metabolism in humans during prolonged exercise. J Appl Physiol 78: 725–735

van Hall G, Saltin B, van der Vusse GJ, Soderlund K, Wagenmakers AJM (1995) Deamination of amino acids as a source for ammonia production in human skeletal muscle during prolonged exercise. J Physiol 489/1: 251–261

Huxtable RJ (1992) Physiological actions of taurine. Physiol Rev 72: 101–163

Huxtable RJ, Laird HE, Lippincott S (1981) Rapid depletion of tissue taurine content by guanidinoethyl sulfonate. In: Schaffer SW, Baskin SI, Kocsis JJ (eds) The effect of taurine on excitable tissues. Spectrum Publications Inc., New York, pp 236–241

King PA (1994) Effects of insulin and exercise on amino acid transport in rat skeletal muscle. Am J Physiol 266: C524–C530

MacLean DA, Graham TE, Saltin B (1994) Branched-chain amino acids augment ammonia metabolism while attenuating protein breakdown during exercise. Am J Physiol 267: E1010–E1022

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265–275

Punna S, Ballard C, Hamaguchi T, Azuma J, Schaffer S (1994) Effect of taurine and methionine on sacroplasmic reticular Ca^{2+} transport and phospholipid methyltransferase activity. J Cardiovasc Pharmacol 24: 286–292

Rennie MJ, Tadros L, Khogali S, Ahmed A, Taylor P (1994) Glutamine transport and its metabolic effects. J Nutr 124: 1503S–1508S

Rosenkranz ER (1995) Substrate enhancement of cardioplegic solution: experimental studies and clinical evaluation. Ann Thorac Surg 60: 797–800

Schaffer SW, Allo S, Mozaffari M (1987) Potentiation of myocardial ischaemic injury by drug-induced taurine depletion. In: Huxtable RJ, Franconi F, Giotti A (eds) The biology of taurine: methods and mechanisms. Plenum Press, New York London, pp 151–158

Suleiman M-S (1994) New concepts in the cardioprotective action of magnesium and taurine during the calcium paradox and ischaemia of the heart. Magnesium Res 7: 295–312

Suleiman M-S, Chapman RA (1993) Changes in the principal free amino acids in the Langendorff perfused guinea-pig heart during cardiac arrest with calcium free or high potassium media. Cardiovasc Res 27: 1810–1814

Suleiman M-S, Fernando C, Dihmis W, Hutter J, Chapman RA (1993) A loss of taurine and other amino acids from ventricles of patients undergoing bypass surgery. Br Heart J 69: 241–245

Suleiman M-S, Moffatt A, Dihmis W, Caputo M, Hutter JA, Angelini GD, Bryan AJ (1997a) Effect of ischaemia and reperfusion on the intracellular concentration of taurine and glutamine in the hearts of patients undergoing coronary artery surgery. Biochim Biophys Acta 1324: 223–231

Suleiman M-S, Dihmis WC, Caputo M, Angelini GD A, Bryan AJ (1997b) Changes in myocardial concentration of glutamate and aspartate during coronary artery surgery. Am J Physiol 272: H1063–H1069

Vasdev S, Whalen M, Ford CA, Longrich L, Prabhkaran V, Parai S (1995) Ethanol and threonine-induced hypertension in rats – a common mechanism. Can J Cardiol 11: 807–815

Authors' address: Dr. M.-S. Suleiman, Bristol Heart Institute, University Department of Cardiac Surgery, Bristol Royal Infirmary, Bristol BS2, 8HW, UK.
Fax 0117 9299737, e-mail: M.S. Suleiman@bristol.ac.uk

Received November 19, 1997