Amino Acids (2005) 28: 337–342
DOI 10.1007/s00726-004-0147-8

Amino Acids
Printed in Austria

Effect of a swim training on homocysteine and cysteine levels in rats

V. Gaume[1,5], H. Figard[1,5], F. Mougin[1,5], J. C. Guilland[2], J. M. Alberto[3], J. L. Gueant[3], D. Alber[4], C. Demougeot[5], and **A. Berthelot[5]**

[1] UFR STAPS, EA 479, Besançon, France
[2] Laboratoire de Physiologie, Faculté de Médecine, Dijon, France
[3] Laboratoire de Pathologie Cellulaire et Moléculaire en Nutrition, INSERM 0014, Faculté de Médecine, Vandoeuvre Nancy, France
[4] Laboratoire de Biochimie, Faculté de Médecine, Besançon, France
[5] Laboratoire de Physiologie et de Pharmacologie-Nutrition Préventive et Expérimentale, EA 479, Faculté de Médecine, Besançon, France

Received August 31, 2004
Accepted November 4, 2004
Published online February 17, 2005;

Summary. The purpose of this study was to investigate the effects of a 8-week of swim training on total plasma homocysteine and cysteine levels in 16 male Sprague-Dawley rats aged 17 weeks. We also evaluated the activity of hepatic cystathionine β-synthase (CBS), an enzyme involved in the metabolism of Hcy, the concentration of plasma glutathione, taurine, and a fraction of vitamin B6: the pyridoxal 5-phosphate (PLP). After one week of acclimatization, rats were randomly divided into two groups: 8 non-trained (NTR) and 8 trained rats (TR). Following the training period, body weight gain was lower in TR than in NTR. Plasma homocysteine did not differ among groups while significantly lower plasma cysteine and taurine levels were found in TR ($157.83 \pm 8.6\,\mu$mol/L; $133.01 \pm 9.32\,\mu$mol/L; $P < 0.05$) compared with data of NTR ($176.19 \pm 4.9\,\mu$mol/L; $162.57 \pm 8.16\,\mu$mol/L; $P < 0.05$). No significant changes in hepatic CBS activity were observed in TR compared with NTR. Moreover, values for plasma glutathione and PLP concentrations were not affected by training.

These results indicate that training reduces plasma cysteine and taurine levels whereas it does not modify other studied parameters. Thus, physical training may regulate cysteine metabolism.

Keywords: Cystathionine β synthase – Cysteine – Glutathione – Homocysteine – Pyridoxal 5′ phosphate taurine – Swim training

Introduction

Homocysteine is a naturally occuring sulfur containing non protein amino-acid. It is either remethylated to methionine, or converted to cysteine in what is known as the transsulfuration patway. The metabolism of homocysteine to cysteine is catabolized by two vitamin B6 dependent enzymes, i.e. cystathionine β-synthase (CBS) which catalyses the condensation of homocysteine with serine to form cystathionine, and cystathionase, which catalyses the hydrolysis of cystathionine to cysteine (Banerjee et al., 2003).

Cysteine is another amino-acid containing sulfhydryl group. Its chemical structure and physicochemical properties are similar to those observed with homocysteine. Cysteine is irreversibly metabolized in liver to yield either glutathione, either taurine, or inorganic sulfate in urine.

Moderately increased concentrations of total homocysteine (tHcy) have been shown to be a strong independent risk factor for cardiovascular disease (Hankey and Eikelboom, 1999), as well as increased concentration of total cysteine (tCys) (Araki et al., 1989; Mansoor et al., 1995; Verhoef et al., 1996).

Physical exercise is reported to lower the incidence of cardiac events (Erikssen et al., 1998). On the contrary, sedentary life is considered to be a risk for cardiovascular diseases (Blair et al., 1995; De Backer, 1998; Hardman, 1996).

To our knowledge, the effects of exercising on plasma homocysteine and cysteine concentrations in rats have not been extensively studied. In addition, few data are available with regard to any metabolic derivates of homocysteine having a potential vascular toxicity. Therefore, we investigated the effects of a 8-week swim training (1 hour/day, 5 days/week) in rats on plasma homocysteine, cysteine, glutathione, taurine and CB, an enzyme which is involved in the metabolism of homocysteine. Moreover, since vitamin B6 is an important cofactor of the transsulfuration

that is frequently responsible for change tHcy and tCys levels, we also measured the pyridoxal 5′-phosphate (PLP; the principal active form of vitamin B6).

Materials and methods

Animals

Sixteen Sprague-Dawley male rats aged 17 weeks and weighing 160–180 g at the beginning of the experiment were used. Rats were fed a standard diet (AO4-SAFE, Villemoison-Epinay-Sur-Orge, FRANCE), and provided with water *ad libitum* for an acclimatization period of one week. They were housed in groups of four in stainless steel cages in a well-ventilated room under controlled environmental conditions temperature ($22 \pm 2°C$), relative humidity (50–60%), and photoperiod (12 h light/dark schedule). After this acclimatization period, rats were randomly divided into two groups: non-trained rats (NTR, $n = 8$) and trained rats (TR, $n = 8$). All rats were inspected daily and weighed once per week.

The research was conducted under the guidelines of the French Ministry of Agriculture for experiments on animals.

Training program

Exercised animals were subjected to a swimming program which had been validated by Guilland et al. (1988). After one week adaptation period, exercise consisted in swimming up to 1 h/d, 5 d/wk, for a total of 8 wks. Animals swam (4 per group) in a plastic container (diameter: 50 cm; depth: 50 cm) filled with water maintained at a temperature of $35 \pm 1°C$. Rats could not touch the bottom or hang on the sides and were supervised during the entire swimming time.

During training, exercise duration was held constant while exercise intensity was increased every week: each rat was loaded with increasing weight (1% of body weight in the first week 1 to 5% in the last week).

Body weight

Body weight of each rat was measured every week. At the end of training, each rat was housed alone in a polyethylene cage for 2 days in order to measure the consumed amount of food and drink.

Collection of blood and tissue

At the end of the experimental protocol, food was withheld overnight. The next morning, 24 hours after the last exercise training period, rats were anaesthetised with pentobarbital (60 mg/kg). Following a midline abdominal incision, blood samples were collected *via* abdominal aorta using 10-ml plastic syringes fitted with 23-gauge needles. Blood samples were immediately centrifuged at $4000 \times g$ for 10 min at 4°C. Plasma was removed and stored at −80°C until analysis. Then, livers were rapidly removed, washed in physiological saline buffer, frozen in liquid nitrogen and stored at −80°C.

Biochemical analysis

Plasma homocysteine, cysteine and glutathione analysis

Total plasma homocysteine, cysteine and glutathione concentrations were measured by HPLC (ESA 580 Kontron instruments 400; Kontron; Strasbourg, France) with fluorometric determination (Bio-Tek, SFM 25; Kontron) after derivation of thiols with 7-fluoro-2,1,3-benzodiazole-4 sulfonamide according to the method described by Durand et al. (1996).

Samples (20 μl) were analysed by HPLC equipped with a fluorescence detector set at λ_{ex} 385 nm and λ_{em} 515 nm. The eluent phase consisted of 0.1 M-KH_2PO_4 buffer (pH 3.25) containing acetonitrile (100 ml/L), and the rate was fixed at 1.2 ml/min. Separation was carried out on a 250×4.6 mm, 5 μm diameter Nucleosil C18 analytical column (Interchim Montluçon France) maintained at 35°C.

Twenty μl of 1.55 M NaOH, 250 L μl of 0.125 M borate buffer (pH 8) and 30 μl of 4.6 mM-7-fluoro-2,1,3-benzoxadiazole-4-sulfonamide solution (in borate buffer) were added to 100 μl filtered supernatant fraction. After derivation at 50°C for 20 min, the samples were rapidly cooled and 40 μl 1 M-HCL were added. After standard calibration of thiols, Hcy, Cys and glutathione concentrations were determined by the area quotient of N-acetylcysteine.

Plasma taurine

Plasma taurine was determined with an amino acid analyser (L-8800, Hitachi, Japon).

Cystathionine beta synthase activity in liver

The enzyme activity was measured by a modified method as described by Shinichi et al. (1998). The assay mixture contained 100 mM Tris HCL, pH 8.6, 1 mM PLP, 1 mM cystathionine (Cth), 5 mM [14 C] serine (~500 cpm/nmoL), 15 mM Homocysteine (Hcy) and 20 μL (~400 μg of total proteins) of homogenate in a final volume of 100 μL. The reaction was incubated for 30 min in a 37°C bath, and stopped by the addition of 25 μL of formic acid. The volume was adjusted to 1 mL with Buffer A (25 mM maleic acid pH 1.8, LiCl 0.2 M). The mixture was centrifuged at 10.000 g for 10 min at RT, and the supernatant loaded onto a 0.8×4 cm column (Bio-Rad Poly-Prep) containing 1 mL of Dowex 50WX2-200 resin from Sigma (equilibrated with 4×5 mL of buffer A). The column was washed with 1 mL, 3 mL and 3×5 mL of buffer A to eliminate the [14C]ser. The [14C]Cth was eluted with 2×3 mL of buffer B (Acetic acid 50 mM, pH 4.8, LiCl 0.8 M) and 3 mL of product was collected in a scintillation vial. The radioactivity was counted by adding 15 mL of Hionic-Fluor (Packard) as scintillation fluid. Negative control was obtained by replacing the homogenate by bovine serum albumin.

Vitamin B6 analysis

Plasma pyridoxal 5′-phosphate (PLP) concentration in plasma extracts was measured using a reverse-phase HPLC method as described by Edwards et al. (1989).

Statistical analysis

Values are expressed as mean ± SEM. The significance of differences between groups was calculated by an one-way analysis of variance (ANOVA) followed by a Tukey-Kramer unpairwise post-hoc test.

The level of significance was set at $p < 0.05$. All analyses were performed using Sigma Stat 2.03.

Results

During the test period, all animals gained weight than the beginning. Moreover, as shown on Fig. 1, from week 7, body weight of TR was significantly lower than the one of NTR. This decrease occured despite the fact that TR consumed more daily food intake than NTR (25.1 ± 0.7 g/day vs. 20.1 ± 1.6 g/day; $P < 0.05$).

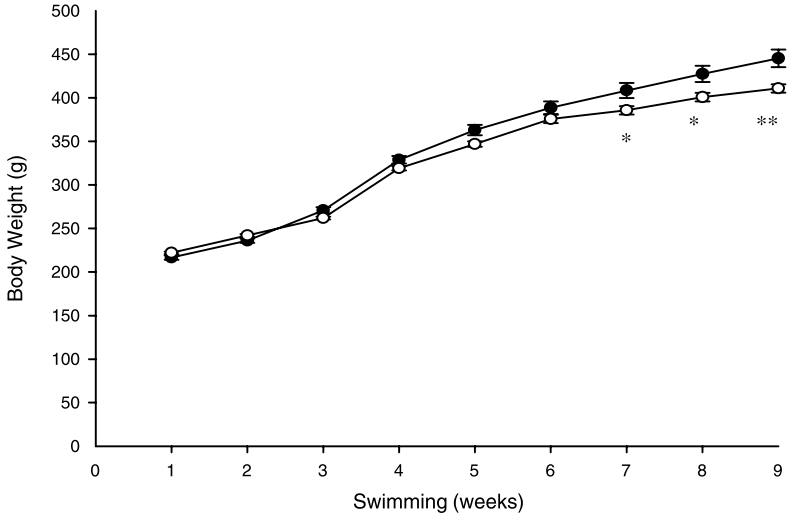

Fig. 1. Effect of exercising on the evolution of the body weight (g) in non-trained (●) and trained rats; n = 8; (○).Values are means ± SEM. Weight for trained group was significantly different from the weight of non-trained group from the 7th week of swimming: $^{*}P < 0.05$; $^{**}P < 0.01$

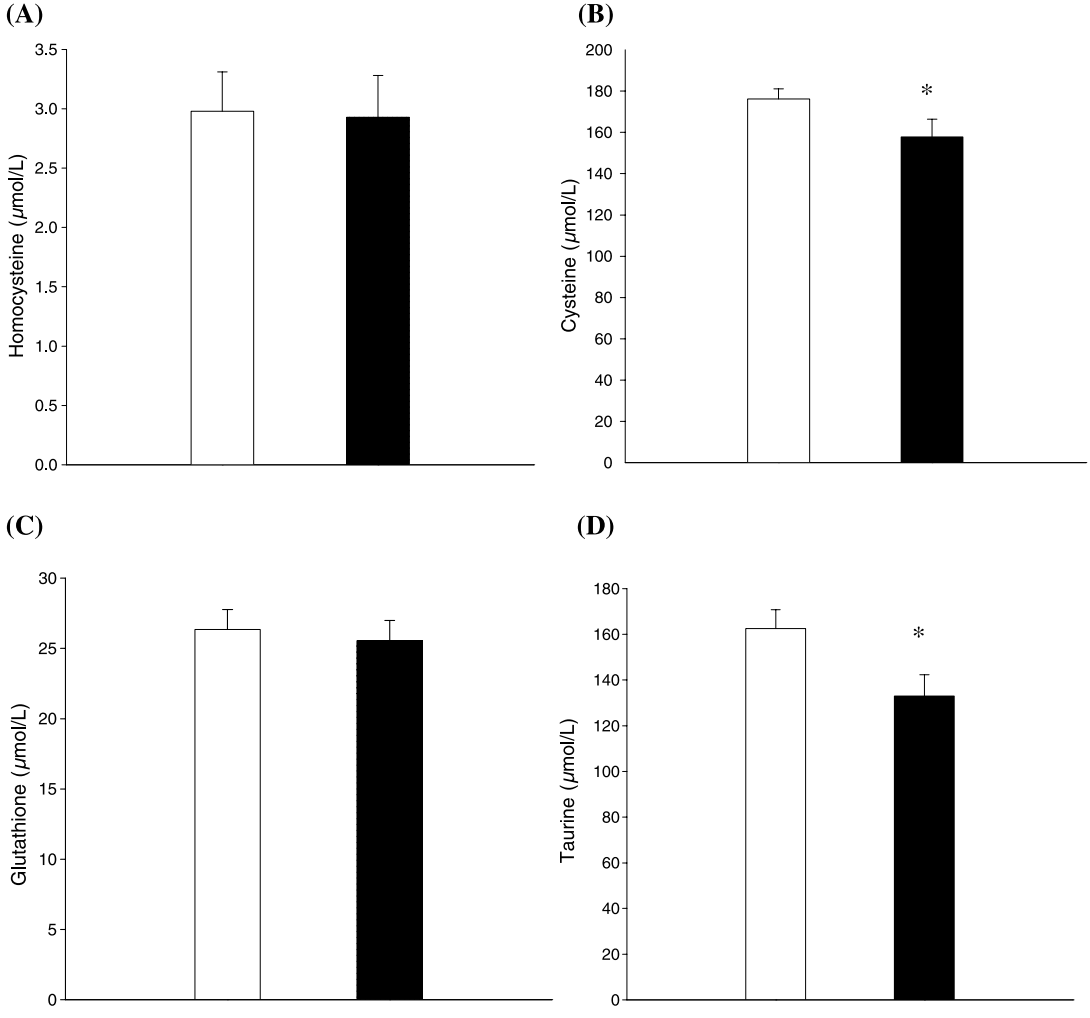

Fig. 2. Total plasma homocysteine (**A**), cysteine (**B**), glutahione (**C**) and taurine (**D**) levels in non-trained and trained rats. Hatched columns represent the values of non-trained rats and dark columns represent the values of trained rats. $^{*}P < 0.05$

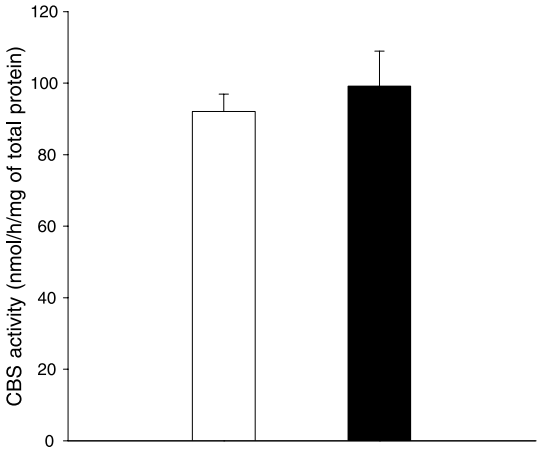

Fig. 3. Cystathionine beta synthase activity liver in non-trained (□) and trained rats (■). Hatched columns represent the values of non-trained rats and dark columns represent the values of trained rats

Following the training period, plasma homocysteine concentrations did not differ among groups ($2.93 \pm 0.35\,\mu$mol/L in TR vs. 2.98 ± 0.33 in NTR μmol/L). Conversely, significantly lower cysteinemia was found in TR group ($157.83 \pm 8.6\,\mu$mol/L) compared with data of NTR ($176.19 \pm 4.9\,\mu$mol/L; $P < 0.05$).

Mean values for plasma glutathione were not significantly different between both groups.

Plasma taurine concentrations appeared to be lower in TR ($133.01 \pm 9.32\,\mu$mol/L) than NTR ($162.57 \pm 8.16\,\mu$mol/L; $P < 0.05$) (Fig. 2).

As shown on Fig. 3, no significant change in hepatic cystathionine β-synthase activity was observed in TR (99.16 ± 9.7 mmol/h/mg of total protein) compared to the values of NTR (92.83 ± 4.89 mmol/h/mg of total protein).

Post-training values for pyridoxal -5 phosphate were not different in TR compared to those of NTR (292.50 ± 24.65 mmol/ vs. 318.87 ± 36.43 mmol/L respectively).

Discussion

The main results of the present study indicated that a 8-week swim training did not change the baseline plasma homocysteine levels in Sprague-Dawley rats despite a significant decrease in cysteine levels. This induced exercise decrease in cysteinemia was associated with a decrease in plasma taurine concentrations. Moreover, trained rats showed identical values in hepatic cystathionine β-synthase (CBS) activity and glutathione levels to those measured in non-trained rats. In addition, swim training did not modify the plasma pyridoxal-5′ phosphate concentrations.

To our knowledge, there are few literature data available on the effects of physical exercise on plasma homocysteine and especially on cysteine levels in rats.

Our observations are in accordance with the results of De Cree et al. (2000) and Wright et al. (1998) who also found in humans no effect of acute, submaximal exercise of relatively short duration on the plasma homocysteine levels.

Other studies reported a decrease in homocysteine levels in healthy men following a resistance training for 8 weeks (Steenge et al., 2001) or in cardiac rehabilitation patients submitted to 12 weeks of aerobic exercise (30–40 min/d) at 70–80% of maximal heart rate (Ali et al., 1998). Furthermore, reduced homocysteine level was observed after a high and low-intensity resistance exercise training in older healthy adults (Vincent et al., 2003). In contrast, Hermann et al. (2003) showed that a high intensity interval training lasting 3 weeks induced an increase (by about 10%) in homocysteine. In the same way, Bailey et al. (2000) reported a 10% increase in resting homocysteine after 4 weeks of cyclo-ergometer training in healthy men.

These discrepancies in data may be caused by different methodologies. In these last findings, the authors investigated either young, or older healthy subjects, who can not be compared with our experimental animals. Moreover, the differences could be explained by the divergent method used in the measurement of plasma tHcy between our study and those described by other laboratories. Indeed, the standardized pre-analytical conditions are necessary for reliable measurement of this plasma thiol. As pointed out by Zighetti et al. (2004), blood samples must be maintained on crushed ice until separation of plasma from blood cells to minimize the artifactual changes.

In addition, gender, age, intensity, duration and amount of training represent as many factors which may explain different values in tHcy. In our study, the training imposed could be insufficient in intensity and duration to induce a decrease in homocysteine.

It is quite well documented that vitamin B6 (pyridoxal 5′-phosphate; PLP) is a cofactor of cystathionine β-synthase (CBS) involved in Hcy metabolism. Homocysteine is converted to cystathionine to form cysteine via the transsulfuration pathway, which is facilitated by two pyridoxal 5′-phosphate-dependent enzymes, cystathionine β-synthase and γ-cystathionase (Cooper, 1983). So, the intake of vitamin B6 in particular plays a central role (Selhub et al., 1993). A vitamin B6 supplementation is responsible for moderate or intermediate basal hypohomocysteinemia and hypercysteinemia (Smolin et al.,

1982). Here, rats were not supplemented with vitamin B6, and no change neither in plasma pyridoxal 5′-phosphate nor in hepatic activity CBS were observed after training. So, this might explain the lack of change in homocysteine levels in trained group.

Studies investigating Hcy in rats after a physical exercise lasting several weeks are inexistant and more data is needed to understand the behavior of Hcy after training.

While effort has been directed to study homocysteine as an important indicator of cardiovascular disease, little attention has been given to cysteine, one the most abundant thiols in plasma which is structurally similar and metabolically linked to homocysteine. Recently, the association of high plasma levels of total cysteine with the risk of thrombosis has been reported (Jacob et al., 1999; Marcucci et al., 2001). On the contrary, no research investigated the effects of exercise program on tCys. In a previous investigation, we reported for the first time that a chronic physical activity induced a decrease in basal plasma tCys concentration in middle-age subjects (Gaume et al., publication in progress). These results are confirmed in this present study in which exercise training was effective in decreasing tCys levels. It is noteworthy to mention that this reduction was found in Sprague Dawley rats with ''normal'' restings values of cysteine. The mechanism by which exercise reduces plasma cysteine levels is currently unknown. Exercise has been shown to affect amino-acid and protein metabolism (Felig and Wahren, 1971; Henriksson, 1991). Although, cysteine metabolism during exercise has not been examined extensively, it is possible that its uptake or production may be influenced by physical activity. The increased metabolic demands of exercise could decrease cysteine and then taurine levels, the taurine being synthesised endogenously from cysteine. Taurine is also a sulfur amino acid with high levels in skeketal muscle (Jacobsen et al., 1968). We may suppose that during exercise, the taurine was more used by skeletal muscle, decreasing its plasma concentration. However, it is not possible to distinguish between increased production or decreased utilisation during exercise. Few articles have referred to the effects of taurine with exercise (Pitkanen et al., 2002; Takekura et al., 1986; Watanabe et al., 1987). Only Matsuzaki et al. (2002) have shown that the taurine concentration in rats skeletal muscles was decreased with exercise. Our paper makes no mention of changes in muscular taurine concentration because it has not been assessed.

The metabolism of cysteine itself is of a large complexity, taking part in various biochemical pathways, therefore, further investigation is needed.

To conclude, this study indicates that training could reduce plasma cysteine and taurine levels whereas it does not modify other studies parameters. Thus, this study suggests that physical training could regulate cysteine metabolism. Nevertheless, the effects of physical exercise in lowering cysteine concentrations require further investigation. These results stress on the importance of studying homocysteine and cysteine levels simultaneously to distinguish their respective roles in prevention of CVD and their relation with physical exercise.

Acknowledgments

We thank Professor Frederic Roussey for helpful English reviewing of the manuscript.

References

Ali A, Mehra MR, Lavie CJ, Malik FS, Murgo JP, Lohmann TP, Li S, Lin HC, Milani RV (1998) Modulatory impact of cardiac rehabilitation on hyperhomocysteinemia in patients with coronary artery disease and ''normal'' lipid levels. Am J Cardiol 82: 1557–1560

Araki A, Sako Y, Fukushima Y, Matsumoto M, Asada T, Kita T (1989) Plasma sulfhydryl containing amino acids in patients with cerebral infarction and in hypertensive subjects. Atherosclerosis 79: 139–146

Bailey DM, Davies B, Baker J (2000) Training in hypoxia: modulation of metabolic and cardiovascular risk factors in men. Med Sci Sports Exerc 32: 1058–1066

Banerjee R, Evande R, Kabil O, Ojha S, Taoka S (2003) Reaction mechanism and regulation of cystathionine B-synthase. Biochimica and Biophysica Acta 1647: 30–35

Blair SN, Kohl HW, Barlow CE, Paffenbarger RS, Gibbons LW, Macera CA (1995) Changes in physical fitness and all-cause mortality. A prospective study of healthy and unhealthy men. JAMA 273: 1093–1098

Cooper AJ (1983) Biochemistry of sulfur-containing amino acids. Ann Rev Biochem 52: 187–222

De Backer (1998) Physical activity and psychological variables in atherosclerosis. Acta Cardiol 29: 107–112

De Cree C, Whiting PH, Cole H (2000) Interactions between homocyst(e)ine and nitric oxide during acute submaximal exercise in adult males. Int J Sports Med 21: 256–262

Durand P, Fortin LJ, Lussier-Cacan S, Davignon J, Blache D (1996) Hyperhomocysteinemia induced by folic acid deficiency and methionine load-applications of modified HPLC method. Clinica Chim Acta 252: 83–93

Edwards P, Liu PKS, Rose GA (1989) A simple liquid chromatographic method for measuring vitamin B6 compounds in plasma. Clin Chem 35: 241–245

Erikssen G, Liestol J, Bjornholt E, Thaulow L, Sandvik L, Erikssen C (1998) Changes in physical fitness and changes in mortality. Lancet 352: 759–762

Felig P, Wahren J (1971) Amino acid metabolism in exercising man. J Clin Invest 50: 2703–2713

Guilland JC, Moreau D, Genet JM, Klepping J (1988) Role of catecholamines in regulation by feeding of energy balance following chronic exercise in rats. Physiol Behav 42: 365–369

Hankey GJ, Eikelboom JW (1999) Homocysteine and vascular disease. Lancet 354: 407–413

Hardman AE (1996) Exercise in the prevention of atherosclerotic, metabolic and hypertensive diseases: A review. J Sports Sci 14: 201–218

Henriksson J (1991) Effect of exercise on amino acid concentrations in skeletal muscle and plasma. J Exp Biol 160: 149–165

Hermann M, Wilkinson J, Schorr H, Obeid R, Georg T, Urhausen A, Scharhag J, Kindermann W, Hermann W (2003) Comparison of the influence of volume-oriented training and high-intensity interval training on serum homocysteine and its cofactors in young, healthy swimmers. Clin Chem Lab Med 41: 1525–1531

Jacob N, Bruckert E, Giral P, Foglietti MJ, Turpin G (1999) Cysteine is a cardiovascular risk factor in hyperlipidemic patients. Atherosclerosis 146: 53–59

Jacobsen JG, Smith LH (1968) Biochemistry and physiology of taurine and taurine derivatives. Physiol Rev 48: 424–511

Mansoor MA, Bergmark C, Svardal AM, Lonning PE, Ueland PM (1995) Redox status and protein binding of plasma homocysteine and other aminothiols in patients with early onset peripheral vascular disease. Homocysteine and peripheral vascular disease. Arterioscler Thromb Vasc Biol 15: 232–240

Marcucci R, Brunelli T, Giusti B, Fedi S, Pepe G, Poli D, Prisco D, Abbate R, Gensini GF (2001) The role of cysteine and homocysteine in venous and arterial thrombotic disease. Am J Clin Pathol 116: 56–60

Matsuzaki Y, Miyazaki T, Miyakawa S, Bouscarel B, Ikegami T, Tanaka N (2002) Decrease taurine concentration in skeletal muscles after exercise for various durations. Med Sci Sports Exerc 34: 793–797

Pitkanen H, Mero A, Oja SS, Komi PV, Rusko H, Nummela A, Saransaari P, Takala T (2002) Effects of training on the exercise-induced changes in serum amino acids and hormones. J Strength Cond Res 16: 390–398

Selhub J, Jacques PF, Wilson PW, Rush D, Rosenberg IH (1993) Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. JAMA 281: 1632–1637

Smolin LA, Benevenga NJ (1982) Accumulation of homocysteine in vitamin B6 deficiency. A model for the study of cystathionine synthase deficiency. J Nutr 112: 1264–1272

Steenge GR, Verhoef P, Geenhaff PL (2001) The effect of creatine and resistance training on plasma homocysteine concentration in healthy volunteers. Arch Intern Med 161: 1455–1456

Takekura H, Tanaka H, Wanatabe M, et al. (1986) Effects of taurine on glycolytic and oxidative enzyme activities of rat skeletal muscles. Sulfur Amino Acids 9: 125–132

Taoka S, Ohja S, Shan X, Kruger WD, Banerjee R (1998) Evidence for Heme-mediated Redox Regulation of Human Cystathionine beta-Synthase Activity. J Biol Chem 273: 25179–25184

Vincent KR, Braith RW, Bottiglieri T, Vincent HK, Lowenthal DT (2003) Homocysteine and lipoprotein levels following resistance training in older adults. Prev Cardiol 6: 197–203

Verhoef P, Stampfer MJ, Buring JE, Gaziano JM, Allen RH, Stabler SP, Reynolds RD, Kok FJ, Hennekens CH, Willet WC (1996) Homocysteine metabolism and risk of myocardial infarction: relation with vitamins B12, and folate. Am J Epidemiol 143: 845–859

Watanabe M, Minato K, Ono M (1987) Effects of taurine on the metabolism under physical exercise. Sulfur Amino Acids 10: 183–186

Wright M, Francis K, Cornwell P (1998) Effect of acute exercise on plasma homocysteine. J Sports Med Phs Fitness 21: 262–265

Zighetti ML, Chantarangkul V, Lombardi R, Lecchi A, Cattaneo M (2004) Effects of some pre-analytical conditions on the measurement of homocysteine and cysteine in plasma. Clin Chem Lab Med 42: 204–207

Authors' address: Vincent Gaume, Laboratoire de Physiologie et de Pharmacologie-Nutrition Préventive Expérimentale, Faculté de Médecine et de Pharmacie, EA 479, Place Saint Jacques, F-25030 Besançon Cedex, France,

E-mail: vincentgaume@hotmail.com