Sport-related hyperhomocysteinaemia: a putative marker of muscular demand to be noted for cardiovascular risk

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Accepted 7 January 2008
Published Online First 23 January 2008

ABSTRACT

Objective: Regular physical activity is associated with a reduction of cardiovascular morbidity and mortality; however, evidence of unfortunate cardiovascular events accompanying elite sport involvement continues to accumulate. To date, no information is available on possible peculiarities of the cardiovascular risk profile in athletes.

Design: The aim of this study was to evaluate plasma homocysteine levels in a group of athletes and to search for relationship with vitamin status and other metabolic variables in order to confirm the existence of a "sport-related hyperhomocysteinaemia" and to explain its clinical significance.

The study population was composed of 82 athletes (59 male and 23 female) practising different sports and 70 healthy age-matched subjects (40 male and 30 female) as a control group. Besides the general clinical and analytical determinations, the assessed variables included homocysteine, folate, vitamin B12, total and high-density lipoprotein (HDL) cholesterol, lactate dehydrogenase (LDH), creatine kinase (CPK) and interleukin-6 (IL-6).

Results: The prevalence of hyperhomocysteinaemia (>15 μmol/l) in athletes and controls was 47% and 15%, respectively. No correlation was found between homocysteine and any of the other investigated variables, in particular plasma folate, blood pressure, LDH, CPK, total and HDL cholesterol and IL-6.

Conclusion: The results of this study confirm the existence of a sport-related hyperhomocysteinaemia which appears linked neither to the same variables found in the general population, nor to specific training-related variables. We suggest that it would represent an adaptation to training but the possibility of a secondary vascular damage cannot be excluded.

Cardiovascular diseases (CVD) represent the leading cause of morbidity and mortality all over the world, causing almost 16 million deaths each year.[1] The main goal of research in recent years has been the comprehension of the underlying risk factors, in view of both primary and secondary prevention strategies. Overwhelming evidence points to the reduction of cardiovascular morbidity and mortality as a function of physical activity.[2] Indeed, regular exercise has shown the capability of reducing chronic inflammation,[3] which plays a key role in the atherogenic process. Importantly, exercise training has been reported to lead to improved endothelium-dependent vasodilatation as a consequence of increased endothelial nitric oxide (NO) synthase (eNOS) activity, which has been identified as a major mechanism explaining the numerous benefits of exercise both in healthy people and in those with different pathological conditions.[4 5] Other mechanisms by which regular physical training reduces cardiovascular risk include the positive effects on blood pressure, body composition, insulin sensitivity and psychological behaviour.[6–11] Unfortunately, an increasing number of cardiovascular deaths in athletes have been reported by different authors over recent decades.[12–14] While many data regarding the causes and the mechanisms of exercise-related sudden cardiac death are available,[14] information about the cardiovascular risk profile of competitive athletes is still poor. A number of studies have documented increased plasma homocysteine (Hcy) levels either as a response to an acute bout of high-intensity exercise or during sustained physical activities.[15–20] We have previously reported a significantly higher percentage of subjects with hyperhomocysteinaemia in winter elite athletes than in the general population.[21] These data are worthy of interest since hyperhomocysteinaemia has been identified as an independent cardiovascular risk factor.[22] In particular, a 5 μmol/l increase in homocysteine concentrations has been judged equivalent to an 0.6 mmol/l rise in total cholesterol as far as the cardiovascular risk is concerned.[23] During recent years, abundant epidemiological evidence has been accumulated demonstrating that even moderate hyperhomocysteinaemia is a strong predictor of cardiovascular morbidity[24 25] and mortality,[26–28] and the risk of coronary artery disease in patients with a Hcy plasma level of more than 15 μmol/l seems to be increased 2.4-fold.[29]

A suggested mechanism by which hyperhomocysteinaemia could induce vascular damage has been recently proposed. A crucial point is represented by endothelial dysfunction,[30] which is mediated through the inhibition of cellular transport of L-arginine,[31] leading to a reduction in NO formation.[32 33] Additionally, raised Hcy concentration leads to increased levels of adhesion molecules and expression of procoagulant species including plasminogen activator inhibitor-1 (PAI-1), tissue plasminogen activator (tPA), protein C (PC) and thrombomodulin. These events may promote platelet aggregation, leucocyte adhesion and thrombosis.[32–36] The effects of homocysteine would be direct (toxic effect) and/or indirect, through the generation of superoxide, hydrogen peroxide and other oxygen-derived free radicals.[37] Finally, recent studies suggest an additional effect of

Hcy-thiolactone in endothelial dysfunction, mediated by protein homocysteinylation.[38]

To date, available data on exercise-related hyperhomocysteinaemia are limited and the clinical significance of this metabolic alteration found in apparently healthy and asymptomatic athletes is still obscure.

The aim of the present study was to evaluate plasma levels of Hcy in a group of competitive, non-professional athletes and to search for relationships with vitamin status and other metabolic variables in order to confirm the existence of a ''sport-related hyperhomocysteinaemia'' and to explain its clinical significance.

MATERIALS AND METHODS

Subjects

The study population was composed of 82 competitive, non-professional athletes (59 male and 23 female, aged 27 (SD 5.38) years) practising different sports disciplines, in particular basketball (n = 27), swimming (n = 40) and soccer (n = 15).

All subjects had been training regularly for almost 1 year, 1–2 hours per day, 3–6 days per week, and most of them had practised the same or other sports in the past.

A group of 70 healthy age-matched subjects (40 male, 30 female), recruited from blood donors, served as controls.

None of the subjects was receiving medication, with the exception of non-steroidal anti-inflammatory drugs if needed. All the subjects were healthy: in particular no abnormal levels of blood pressure, no abnormal liver or renal function, no history of diabetes or CVD was found. Moreover, all athletes, through the compilation of an anonymous questionnaire, denied the consumption of any prohibited substances.

Athletes were instructed to abstain from caffeine, alcohol and drug consumption and to refrain from any strenuous physical activity for 24 h before the examination, which consisted of a blood sampling in the morning (08:00 h, after an overnight fast) and a medical evaluation including familial and personal medical history, a detailed physiological and sportive personal history and a complete physical examination.

The study was designed in agreement with the Declaration of Helsinki and was approved by the local Ethical Committee. The athletes and control subjects volunteered for the study and gave their informed consent.

Assays

Total cholesterol, high-density lipoprotein-cholesterol (HDL-C), triglyceride, and creatine kinase (CK) concentrations were measured spectrophotometrically by the clinical chemistry Aeroset analyser (Abbott Laboratories, Abbott Park, Illinois, USA). Ferritin, Vitamin B12 and folate were measured by the chemiluminescence immunoassay analyser Architect i 2000 (Abbott Diagnostics Laboratories, Abbott Park, Illinois, USA). Haemoglobin and haematocrit were analysed using an automated Cell Dyn 4000 series analyser (Abbott Diagnostics Laboratories, Abbott Park, Illinois, USA). Total homocysteine level was measured by HPLC – Agilent 1100 series (Bio-Rad Laboratories GmbH, Munchen, Germany). Hyperhomocysteinaemia was defined as plasma Hcy level exceeding 15 μmol/l,[39 40] and has been graded as mild when Hcy in the fasting state is between 15 and 30 μmol/l, moderate when it ranges from 30 to 100 μmol/l and severe when it exceeds 100 μmol/l.[41]

Venous blood samples, drawn into k3-containing tubes, were immediately placed on ice, centrifuged at 2200 rpm at 4°C for 20 minutes and immediately analysed.

Interleukin-6 (IL-6) was determined by high-sensitivity IL-6 ELISA (Diaclone) in serum. Activity of the enzyme was stopped by 1 mol/l phosphoric acid and absorbance was measured by spectrophotometer using 450 nm as the primary wavelength.

All samples were processed in duplicate in the same assay session. Intra-assay and interassay coefficients of variation (CVs) for all the abovementioned assays were below 5%.

Analysis of the MTHFR genotype

Detection of the 5,10-methylene-tetrahydrofolate reductase (MTHFR) C677T polymorphism was proposed to all athletes but only 50% of them gave their consent for the genetic study. Therefore MTHFR polymorphism, studied in all of the control subjects, was tested in only 40 athletes. The DNA source was peripheral blood lymphocytes, and genomic DNA was extracted by conventional methods immediately after the blood drawing. The analysis was carried out using a restriction procedure reported elsewhere[42] and the three genotypes of MTHFR were defined as follows: CC, normal homozygous; CT, heterozygous and TT, mutant homozygous.

Statistical analysis

Database management and all statistical analyses were performed using the Statistica 6 for Windows software package (Statsoft Inc., Tulsa, OK). Normality of data was assessed by the Wilk–Shapiro test. For continuous variables, differences were analysed by means of the two-tailed Student t test when data were normally distributed and using the Mann–Whitney U test when data were nonparametric.

The analysis of variance techniques were performed to evaluate whether alcohol and tobacco use can significantly shift the mean values.

Tests concerning difference among proportions were performed to evaluate the prevalence of high levels of homocysteine in male and female athletes and control groups.

RESULTS

Plasma Hcy levels were significantly higher in athletes than in controls (19.30 (SD 13.72) μmol/l versus 11.97 (SD 3.9) μmol/l) (table 1).

Among the athletes, the percentages of subjects with normal and elevated homocysteine levels, defined by levels below or above the upper limit of the normal range (15 μmol/l), were 53% and 47%, respectively. In the control group, relevant percentages were 85% and 15%, respectively (table 1).

As shown in table 1, the comparison between plasma Hcy of male and female athletes showed significantly higher levels in the former than in the latter group (male, 21.79 (SD 15.2) μmol/l vs female, 12.93 (SD 5.14) μmol/l, $p<0.001$), as well as a higher proportion of individuals with hyperhomocysteinaemia (male, 54% vs female, 30%, $p<0.001$). In particular, 32/59 male athletes showed hyperhomocysteinaemia, with the following distribution: 75% moderate increase, 25% intermediate increase, while 7/23 female athletes presented hyperhomocysteinaemia, all of them with a moderate increase. None of the athletes presented a severely increased level (>100 μmol/l) of Hcy. Also, in our control subjects, Hcy levels were significantly higher in men than in women (13.4 (SD 4.0) μmol/l vs 11.7 (SD 4.3) μmol/l, $p<0.01$) as well as the proportion with hyperhomocysteinaemia (male 20%, female 10%).

Hyperhomocysteinaemia was observed in all the sports disciplines: in particular 12 cases were described in basketball, 16 cases in swimming and 11 cases in soccer.

Table 1 Values of plasmatic homocysteine and prevalence of hyperhomocysteinaemia in athlete and control groups

	Total			Male			Female		
	Athletes n = 82	Control n = 70	Statistical analysis	Athletes n = 59	Control n = 40	Statistical analysis	Athletes n = 23	Control n = 30	Statistical analysis
Homocysteine (μmol/l) (SD)	19.30 (3.72)	11.97 (3.9)	p = 0.000*	21.79 (15.2)	13.4 (4.0)	p = 0.000*	12.93 (5.14)	11.7 (4.3)	NS*
Subjects with hyperhomocysteinaemia	39/82	11/70	p<0.05**	32/59	8/40	p<0.05**	7/23	3/30	p<0.05**

*ANOVA test
**Test for difference between two proportions

In table 2 the mean values of creatine kinase (CK), lactate dehydrogenase (LDH), total cholesterol, high-density lipoprotein-cholesterol (HDL-C), triglycerides (TG), creatinine (CR), urea, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (GGT), vitamin B12, folate and IL-6 of the athletes are also shown. With the exception of 14 athletes who presented mildly increased total cholesterol levels (>200 mg/dl), in two cases associated with a mild increase of plasma triglycerides, the other athletes with hyperhomocysteinaemia presented a normal lipidaemic profile. All of them showed the other investigated variables within the normal range and presented normal levels of blood pressure and heart rate.

No significant differences were found between athletes with normal Hcy levels and those with hyperhomocysteinaemia when considering all the tested parameters. As shown in figs 1 and 2, the statistical analyses showed no significant correlation between Hcy and any of the other investigated variables in either the male or the female group, with particular regard for serum folate, CPK, LDH, HDL-C, vitamin B12, tobacco consumption and alcohol intake, which are known to influence Hcy levels. Serum IL-6 was investigated in order to analyse whether a generalised inflammatory status, linked to a stressful training or competitive condition, could explain the observed increase in Hcy levels. Only one female and four male athletes showed IL-6 levels exceeding the threshold limit (3.59 pg/ml). The observed mean levels of IL-6 were 1.12 (SD 4.8) pg/ml, and again the statistical analyses showed no significant correlation between homocysteine and IL-6 levels in either male or female athletes.

The genetic analysis of the C677T polymorphism in the MTHFR gene showed the following percentage distribution: normal homozygous (CC) 72%, heterozygous (CT) 13.4%, and mutant homozygous (TT) 14.6% in the athlete group and CC 36%, CT 54% and TT 10% in the control group.

DISCUSSION

The result of the present study confirms previous observations of the existence of a "sport-related" hyperhomocysteinaemia. In a group of 82 competitive athletes supranormal Hcy plasma levels were found in 39, corresponding to a prevalence of 47%. We initially described this metabolic alteration in a group of 103 athletes competing in winter disciplines.[21] The prevalence of hyperhomocysteinaemia in this sample was 32%. Therefore, considering also this additional series of 82 competitive athletes, the prevalence of "sport-related" hyperhomocysteinaemia appears to be 45%, significantly higher than in the general population.[39 43]

It is well-known that the leading cause of death in elderly people practising sport is coronary heart disease.[44 45] In the light of this consideration, the comprehension of both the mechanisms and the possible consequences of "sport-related" hyperhomocysteinaemia appears to be of value for preventive strategies.

All athletes involved in the present study showed a low-risk cardiovascular profile, on the basis of the conventional risk factors.[1] Complementary findings can be viewed by low serum IL-6, which has been recognised as a predictor of future cardiac events in asymptomatic, apparently healthy people.[46]

We have found a high prevalence of hyperhomocysteinaemia in two groups of athletes participating in sports that require very different environmental training conditions and with different levels of performance, since the first group was composed of Olympic athletes[21] while subjects involved in the present study were competing in regional or national championships. This likely suggests that climatic training conditions, different aerobic performances, and different training engagement do not influence plasma Hcy levels in athletes.

Table 2 Athletes' analytical data

	Total (n = 82)	Male (n = 59)	Female (n = 23)	p<0.05 male vs female
Urea (mg/dl)	31.68 (7.20)	33.15 (6.8)	27.9 (6.8)	p = 0.002*
Creatinine (mg/dl)	1.05 (0.11)	1.06 (0.1)	1.0 (0.13)	p = 0.028*
AST (U/l)	23.13 (6.72)	24.5 (7.01)	19.6 (4.3)	p = 0.002*
ALT (U/l)	21.15 (9.18)	22.6 (9.5)	17.3 (7.1)	p = 0.018*
GGT (U/l)	19.21 (12.83)	21.2 (14.2)	14.9 (6.6)	p = 0.045*
Total cholesterol (mg/dl)	173.26 (32.60)	170.1 (29.0)	181.4 (40.0)	NS*
HDL cholesterol (mg/dl)	54.30 (13.35)	49.5 (9.5)	66.6 (14.2)	p = 0.000*
TG (mg/dl)	69.00 (42.83)	96.0 (37.4)	56.4 (25.9)	p = 0.000*
IL-6 (pg/ml)	1.12 (4.8)	1.23 (5.5)	0.75 (1.5)	NS*
CPK (U/l)	212.77 (167.68)	249.5 (182.2)	118.6 (55.8)	p = 0.001*
Haemoglobin (g/dl)	14.30 (1.13)	14.8 (0.79)	13.1 (1.02)	p = 0.000*
LDH (U/l)	183.07 (29.23)	187.7 (29.4)	171.3 (25.8)	p = 0.00*
Vitamin B12 (pg/ml)	449.42 (103.81)	432.92 (88.46)	493.68 (128.94)	p = 0.017*
Folic acid (ng/ml)	7.42 (1.99)	7.08 (1.79)	8.32 (2.22)	p = 0.010*
Homocysteine (μmol/l)	19.30 (13.72)	21.79 (15.2)	12.93 (5.14)	p = 0.008*
Proportion of athletes with hyperhomocysteinaemia	39/82	32/59	7/23	p<0.05**

Results expressed as mean (SD) unless otherwise stated.
*ANOVA test; **Test concerning difference between two proportions.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; CPK, creatine kinase; GGT, γ-glutamyl transpeptidase; HDL, high-density lipoprotein; IL-6, interleukin-6; LDH, lactate dehydrogenase; TG, triglycerides.

Furthermore, plasma Hcy levels were increased independently of CK and LDH levels, suggesting that the duration and the intensity of the muscle workload cannot explain this finding.

All athletes with increased plasma Hcy showed no deficiency of plasma vitamin B12 or folate. Thus, neither a dietary lack of these cofactors, which represents the main cause of hyperhomocysteinaemia in the general population,[29] nor a training-related increased utilisation of them could explain this condition.

The C/T polymorphism of the MTHRF gene was studied in 50% of the enrolled subjects. The incidence of TT mutation was in balance with that observed in the control group and both of them were comparable to those described in the general population.[47] Therefore MTHFR TT mutation cannot itself explain the observed prevalence of hyperhomocysteinaemic athletes.

An intriguing explanation of "sport-related" hyperhomocysteinaemia could be linked to muscle tissue metabolism. Hcy is

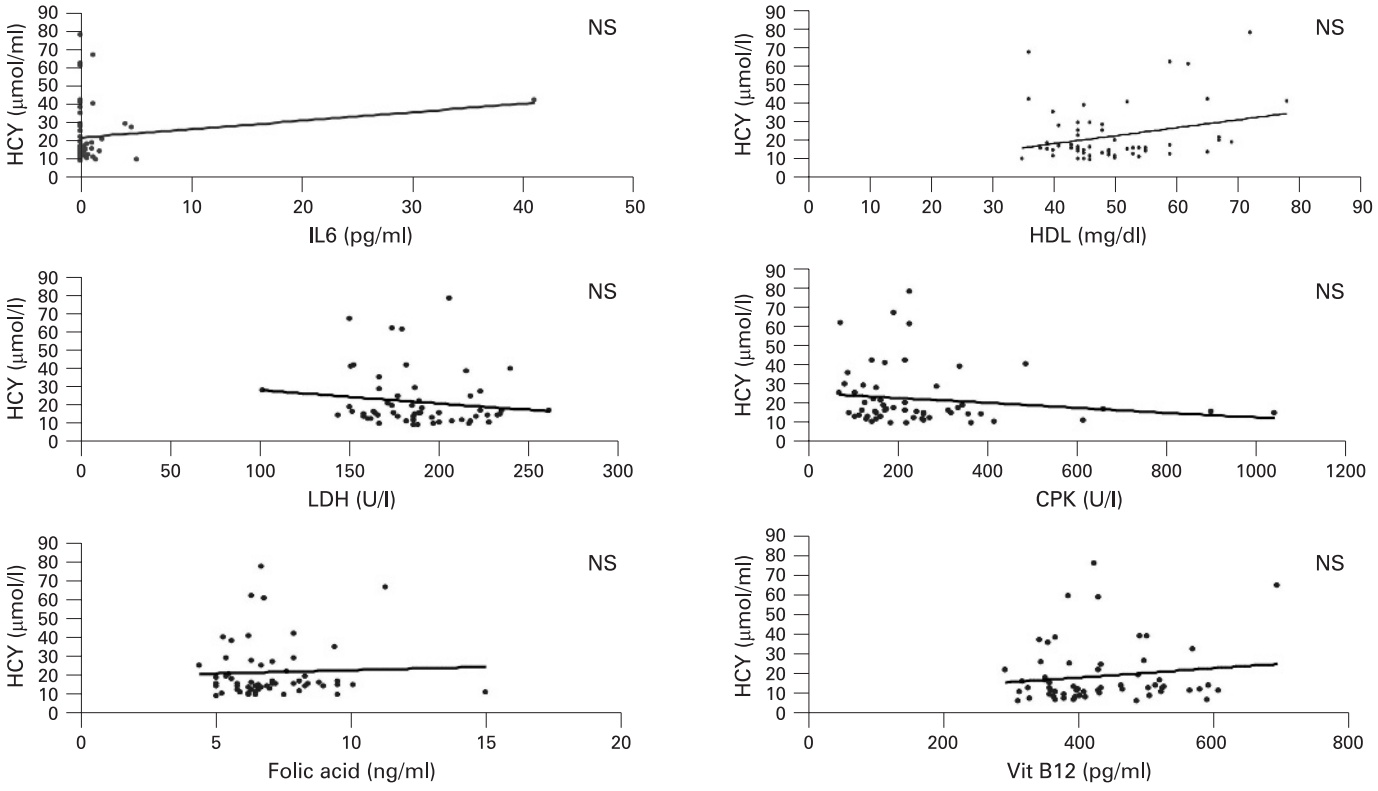

Figure 1 Distribution on a Cartesian plane of the measured values of homocysteine and other parameters (IL-6, HDL, LDH, CPK, folic acid and vitamin B12) in male athletes. CPK, creatine kinase; HCY, homocysteine; HDL, high-density lipoprotein; IL-6, interleulcin-6; LDH, lactate dehydrogenase.

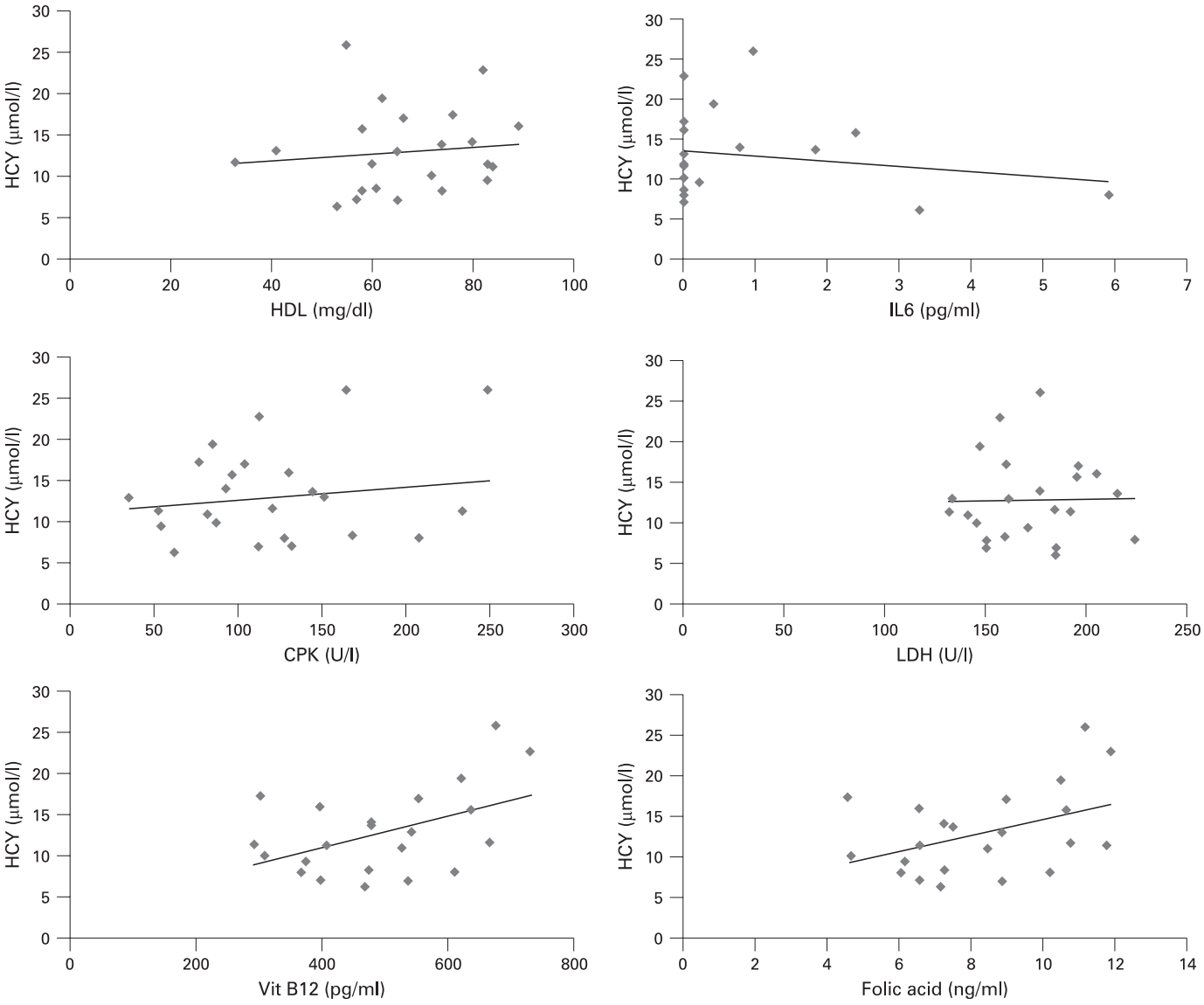

Figure 2 Distribution on a Cartesian plane of the measured values of homocysteine and other parameters (IL-6, HDL, LDH, CPK, folic acid and vitamin B12) in female athletes. CPK, creatine kinase; HCY, homocysteine; HDL, high-density lipoprotein; IL-6, interleulcin-6; LDH, lactate dehydrogenase.

an essential intermediate in the synthesis of the two amino acids methionine and cysteine.[48 49] Prolonged and intense physical exercise conceivably leads to a partial damage of muscle proteins, which have to be resynthesised by muscle cells with the enhancement of the metabolic pathways for the amino acid generation. The enhanced turnover of those crucial amino acids could call for increased Hcy production and export from cells to circulation.[50] In this view, hyperhomocysteinaemia could be interpreted as a marker of metabolic adaptation to training and a positive sign of muscle recovery rather than a pure risk factor for cardiovascular diseases. In fact, Hcy levels significantly increase after a single bout of high-intensity exercise, even in athletes with normal resting values.[17]

The mechanisms by which Hcy impairs endothelial functions are not fully understood.[32 37 51] It has been suggested that Hcy increases superoxide production and decreases NO bioavailability by causing auto-oxidation and by impairing function of the L-arginine transport.[31]

Physical exercise, on the other hand, conceivably attenuates or even minimises Hcy-induced endothelial dysfunction through the increase in both eNOS protein content and activity,[4 5] among a number of beneficial effects on the cardiovascular system.[2 3 52–54]

If this were the case, hyperhomocysteinaemia in athletes could represent a marker of structural demand for muscles but would not actually increase cardiovascular risk. This remains, however, a speculative hypothesis, since specific markers of endothelial damage have not been studied in hyperhomocysteinaemic athletes. Even if the clinical significance of hyperhomocysteinaemia in athletes remains uncertain and all athletes presented folate levels within the normal range, oral folate and B group vitamin supplementation was prescribed to all subjects presenting this alteration, following the current guidelines.[25] Actually, the effectiveness of this therapy in reducing the risk of cardiac events, as a consequence of lowered serum Hcy, has been demonstrated only in patients with heart disease[23] and not in asymptomatic subjects. However, folate supplementation appeared to be justifiable in hyperhomocysteinaemic athletes because of the absence of adverse effects when folic acid is administered in doses between 0.2 and 15 mg/day.[55]

What is already known on this topic

- Several studies have suggested an increase of cardiovascular risk following elite sport participation, and evidence continues to accumulate of unfortunate cardiovascular events that may accompany elite sport involvement. However, no information is available about possible peculiarities of the risk profile in elite active athletes.
- Abundant epidemiological evidence has demonstrated that even moderate hyperhomocysteinaemia is a strong predictor of cardiovascular morbidity and mortality. Different authors described a significant increase in homocysteine (Hcy) plasma levels both in response to an acute bout of high-intensity exercise and in chronic adaptation to physical exercise. We previously described this metabolic alteration in a group of elite athletes competing in winter disciplines. In this setting, plasma Hcy levels were increased independently of the different situations of a sport season as well as vitamin status.

What this study adds

This study confirms the existence of an "exercise-related" hyperhomocysteinaemia in athletes participating in sports that require very different environmental training conditions and with different levels of performance. This likely suggests that climatic conditions, different aerobic performances, and different training engagement do not influence the increased Hcy plasma levels of athletes. Furthermore, plasma Hcy levels were increased independently of CK, LDH and vitamin levels, suggesting that the duration and the intensity of the muscle workload as well as nutritional status cannot explain this finding, which appears not to be linked to the same variables as found in the general population.

To conclude, the results of this study confirm the existence of a sport-related hyperhomocysteinaemia. This appears as a common condition, being present in almost 40–50% of the Italian athletes. Causes and clinical significance of the athletes' hyperhomocysteinaemia remain obscure. There are no apparent relationships with the variables described in the general population.[56–58] Moreover, the type of sport, the level of performance, the muscle workload or peculiar stressful conditions do not seem to influence this metabolic alteration.

Hyperhomocysteinaemia is a cardiovascular risk factor in the general population.[25 28] A different significance in athletes is worthy of attention. We suggest that hyperhomocysteinaemia reflects the adaptation to training, being an expression of the enhanced protein synthesis in muscle cells. Clearly, studies are required to investigate the natural course, as well as the long-term effects, of sport-related hyperhomocysteinaemia. In fact, a major limitation of our previous and present studies is the lack of longitudinal data. Yet, the priority was to investigate established variables that could explain the intriguingly high prevalence of hyperhomocysteinaemia in elite and non-elite athletes of different disciplines. We believe that this has been accomplished with evidence enough to progress further.

Acknowledgements: We thank Eng Stefano Perrini for his expert statistical advice. The authors declare that in this work there are no professional relationships with companies or manufacturers who will benefit from the results of the present study. The authors state that the results of the present study do not constitute endorsement by ACSM.

Funding: This study was supported by a grant from the Ministry of Health of Italy – Commission for the Surveillance of Doping (CVD).

Competing interests: None declared.

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CORRECTION

There was an error in the pagination of the articles published in the October and November 2008 issues of the journal. Please see a corrected list of citations below. The journal apologises for this error.

Khan KM. Preventing ACL injuries, turning research into practice and avoiding media ambush. *Br J Sports Med* 2008;**42**:483–4. should be **Khan KM**. Preventing ACL injuries, turning research into practice and avoiding media ambush. *Br J Sports Med* 2008;**42**:783–4.

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Sport-related hyperhomocysteinaemia: a putative marker of muscular demand to be noted for cardiovascular risk

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Br J Sports Med 2008 42: 894-900 originally published online January 23, 2008
doi: 10.1136/bjsm.2007.045021