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Relationship between homocysteine and cardiorespiratory fitness is sex-dependent

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Abstract

Elevated plasma homocysteine is recognized as an independent risk factor for cardiovascular disease. Recently, there have been conflicting reports of the relationship between physical activity and homocysteine. A more objective measure of physical activity is cardiorespiratory fitness; however, its relationship with homocysteine has yet to be investigated. The aim of this study was to determine the relationship between cardiorespiratory fitness and plasma homocysteine. Cross-sectional associations between cardiorespiratory fitness (VO_2max) and plasma homocysteine were examined in 49 men and 11 women. A submaximal bicycle test was used to determine VO_2max and plasma homocysteine was measured using high performance liquid chromatography with fluorescence detection. Dietary analysis determined B vitamin intake. There was a significant inverse relationship between plasma homocysteine concentration and VO_2max in women ($r = -0.81$, $P = 0.003$) but not in men ($r = -0.09$, $P = 0.95$). There were no significant relationships between plasma homocysteine and age, BMI, body fat, total cholesterol, and LDL cholesterol. In summary, elevated cardiorespiratory fitness is associated with decreased plasma homocysteine concentrations in women.

Keywords: Homocysteine; Cardiovascular disease; Cardiorespiratory fitness; Exercise; Physical activity; Aerobic capacity

1. Introduction

Elevated plasma concentrations of homocysteine are associated with an increased incidence of cardiovascular disease [1]. From a public health perspective, it is important to

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identify modifiable factors that influence homocysteine. Low dietary intake of folate is known to be the major factor elevating plasma homocysteine with lifestyle factors such as smoking and coffee consumption, also associated with increased homocysteine concentrations [2].

A number of large epidemiological studies have reported the relationship between self-reported physical activity and homocysteine with conflicting results. The Hordaland Homocysteine Study [2] reported a significant inverse relationship between homocysteine concentration and physical activity levels after adjusting for intake of vitamin supplements, fruits, and vegetables. In this study on 12,000 subjects the investigators concluded that the effect of physical activity on homocysteine may help to explain the beneficial effect of exercise on coronary risk.

A study of Chinese men and women [3] also reported the same negative relationship; however, after adjusting for dietary vitamin B intake, this association was not significant. The Supplementation with Antioxidant Vitamins and Minerals Study found the relationship to be significant only in men [4], and a study in adults in The Netherlands reported no relationship in men and a weak positive association in women [5]. In all of these studies, questionnaires were used to quantify physical activity; however, it is well known that these instruments have limited validity and reliability. [6–8]. Because of the importance of this issue, the use of a more objective measure of physical activity level is required to determine whether there is a relationship between physical activity and homocysteine.

The determination of cardiorespiratory fitness or maximal oxygen uptake (VO_2max) by validated, well accepted laboratory techniques is used in exercise science as the gold standard for assessing a persons level of physical activity. In this present study, cardiorespiratory fitness was measured using a laboratory-based submaximal VO_2max test to investigate the relationship with plasma homocysteine concentrations.

2. Methods and materials

2.1. Subjects

A total of 60 individuals (49 men and 11 women) volunteered for this study. Each person provided written informed consent to participate in the study, which was approved by the Ethics Committee of the University of Tasmania. Subjects were chosen to represent a wide range of cardiorespiratory capacities including highly trained athletes. Exclusion criteria were as follows: 1) regular cigarette smoking currently or in the past 2 years, 2) known cardiovascular disease, 3) more than two modifiable cardiovascular risk factors according to the criteria of the American Heart Association [9], 4) more than one cup of coffee per day, and 5) use of vitamin B supplements. Characteristics of the study subjects are shown in Table 1.

2.2. Overview of procedures

Each subject attended the Human Performance Laboratory at the University of Tasmania on two separate occasions. During the first visit a fasting blood sample was supplied and

Table 1
Selected characteristics of the subjects

Characteristic	All (n = 60)	Men (n = 49)	Women (n = 11)	P Value (Men vs Women)
Age (y)	29.5 ± 9.3	30.2 ± 9.9	26.4 ± 6.0	0.22
Homocysteine (μmol/L)	9.7 ± 2.3	9.8 ± 2.4	9.4 ± 2.1	0.69
VO_2max (mL/kg/min)	51.4 ± 12.0	50.0 ± 11.2	58.1 ± 14.0	0.04*
Body fat (%)	14.4 ± 6.9	13.1 ± 6.5	20.0 ± 6.7	<0.01*
BMI (kg/m^2)	23.9 ± 2.8	22.2 ± 0.8	24.3 ± 1.4	0.02*
Total cholesterol (mmol/L)	4.7 ± 1.1	4.7 ± 1.2	4.7 ± 0.8	0.84
LDL cholesterol (mmol/L)	2.8 ± 0.9	2.9 ± 1.0	2.5 ± 0.7	0.28
HDL cholesterol (mmol/L)	1.4 ± 0.3	1.3 ± 0.2	1.8 ± 0.2	<0.001*
Folate intake (μg)	407 ± 214	418 ± 212	354 ± 229	0.44
Vitamin B_{12} intake (μg)	4.7 ± 2.2	4.8 ± 2.1	4.5 ± 2.9	0.74
Vitamin B_6 intake (mg)	2.6 ± 1.3	2.7 ± 2.0	1.3 ± 1.1	0.21

Data are mean ± SD.
* Difference in means is significant ($P < 0.05$) with a two-tailed independent t test.

analyzed for plasma homocysteine and lipid profile. Subjects completed medical history and lifestyle surveys as well as the Harvard University semiquantitative food frequency questionnaire. At the second visit, (≤5 days after the first), subjects had anthropometric measures taken and performed the Astrand submaximal cycle ergometer test to determine VO_2max.

2.3. Blood samples and storage

Blood samples (20 mL) were drawn from subjects, via venepuncture at the antecubital fossa into lithium–heparin vacutainers after an overnight fast. Samples were immediately placed on ice before centrifugation at 2000 × g for 5 minutes at 4°C. Plasma was separated and stored at −80°C for later analysis. This process was completed immediately to avoid the release of homocysteine from erythrocytes.

2.4. Measurement of homocysteine

Total plasma homocysteine concentrations were determined using high-performance liquid chromatography (HPLC) with fluorescence detection (Waters Alliance 2696 Separations Module with Waters 470 scanning fluorescence detection, Milford, MA) using a modification of a previously published method [10]. The analyses took place in the biochemistry laboratory at the Defence Nutrition Research Centre. The laboratory is a member of the European Research Network for Evaluation and Improvement of Screening, Diagnosis and Treatment of Inherited Metabolic Disorders external quality assurance program for plasma homocysteine analysis.

Total plasma homocysteine is defined as the sum of all homocysteine species in plasma including homocysteine, mixed disulfides, and protein-bound forms. All of these forms were converted to homocysteine by reduction of sulfide bonds with sodium borohydride. The thiol group was labeled with a fluorescent moiety (monobromobimane) and separated from other

thiols by reverse-phase HPLC after deproteination by perchloric acid. Homocysteine concentrations were calculated by use of an internal standards ratio method against homocystine and cysteamine standards. Coefficient of variation (CV) data from in-house pooled plasma control material ($\sim$10 μmol/L) has demonstrated within-batch CV to be $<$4% and between-batch CV to be $<$3.5%.

2.5. Lipid profiles

Lipid profiles (total cholesterol, HDL cholesterol, and triglycerides) were determined using commercial cholesterol kits (Trace Scientific, Melbourne, Australia) with an automated spectrophotometer (Technicon RA 100, Miami, FL). LDL cholesterol was calculated using the Friedewald equation [11].

2.6. Anthropometric measures

Height and body mass were determined using a calibrated clinical stadiometer and scales, with subjects wearing a bathing suit. Body fat percentages were measured using skinfold callipers (Harpenden, USA), with the sum of three skinfolds and skinfold prediction equations for men [12] and women [13].

2.7. Cardiorespiratory fitness

Cardiorespiratory fitness was determined using the 6-minute submaximal Astrand-Rhyming bicycle ergometer test [14]. The decision to use the submaximal rather than a maximal test was based on high correlation between the two tests (as described below), and on the unavailability of the metabolic system for all 60 individuals. The Astrand-Rhyming protocol is a single-stage test that uses a nomogram to predict VO_2max from heart rate response to one 6-minute workload. We determined the validity of this test in a pilot study ($n = 12$) in which subjects also had their VO_2max determined during a maximal bike protocol with expired air gas analysis (Quinton Metabolic Cart, Quinton, Bothell, WA). The correlation between the two tests was $r = 0.88$, $P < 0.001$. The coefficient of variation of the Astrand-Rhyming test was also determined in the pilot study at 3%.

2.8. Nutrient intake

Dietary intakes of folate, vitamin B_6, and vitamin B_{12} were assessed using the Harvard University semiquantitative food frequency questionnaire. This survey has been shown to be valid and reproducible [15].

2.9. Statistical analyses

The study was designed and powered to determine the relationship between cardiorespiratory fitness in all individuals. For this analysis 60 individuals were required to detect that

Table 2
Correlation coefficients for plasma homocysteine with selected variables

Variable	*R* Value (All)	*R* Value (Men)	*R* Value (Women)
VO_2max (mL/kg/min)	−0.23	−0.09	0.81*
Age	0.09	0.05	0.40
BMI (kg/m^2)	0.09	0.10	−0.13
Body fat (%)	0.04	−0.01	0.49
Total cholesterol (mmol/L)	0.02	0.05	0.21
LDL cholesterol (mmol/L)	−0.19	−0.01	−0.15
HDL cholesterol (mmol/L)	0.24	0.35	0.21
Folate intake (μg)	−0.32*	−0.28	−0.38
Vitamin B_{12} intake (μg)	−0.24	−0.32	−0.11
Vitamin B_6 intake (mg)	−0.34*	−0.33*	−0.38

* Correlation is significant ($P < 0.05$).
BMI = body mass index.

a correlation coefficient of 0.35 was significant with power of 0.8 and α of 0.05 (two sided). The subsequent analysis of data for only the women achieved a β of 0.91 with an α of 0.05.

Plasma homocysteine concentrations were normally distributed for all comparisons. Pearson's correlation coefficients and multivariate analyses were performed between all likely determinants of plasma homocysteine concentration. Variables were divided into tertiles as follows: 1) <33rd percentile; 2) 33rd to 67th percentile; and 3) >67th percentile. Data are expressed as mean ± SD. The SPSS version 11.0.0 software (SPSS, Inc., Chicago, IL) was used for all statistical methods. A value of $P < 0.05$ was considered to be statistically significant.

3. Results

Women had significantly greater ($P < 0.05$) cardiorespiratory fitness, percentage of body fat, BMI, and HDL cholesterol than men (Table 1). Pearson correlation coefficients between independent variables and plasma homocysteine concentrations are shown in Table 2. There was a significant inverse relationship ($r = -0.81$, $P = 0.003$) (Fig. 1) between cardiorespiratory fitness (VO_2max) and plasma homocysteine in women. This association was not found in men ($r = -0.09$, $P = 0.95$) or in analyses of men and women combined ($r = -0.23$, $P = 0.08$) (Fig. 2). Multiple regression confirmed that with all variables included the relationship between cardiorespiratory fitness and homocysteine was not significant ($P = 0.19$). When correlations were adjusted for vitamin B intake, these correlations were still significant for women and were nonsignificant for men as well as for men and women combined (data not shown). As expected, there were significant associations between dietary intakes of folate, vitamin B_6, and vitamin B_{12} in both men and women (data not shown). The inverse relationship between dietary folate intake and homocysteine was significant in all individuals ($r = -0.32$, $P = 0.03$; for men, $r = -0.28$, $P = 0.06$; and for women, $r = -0.38$, $P = 0.08$).

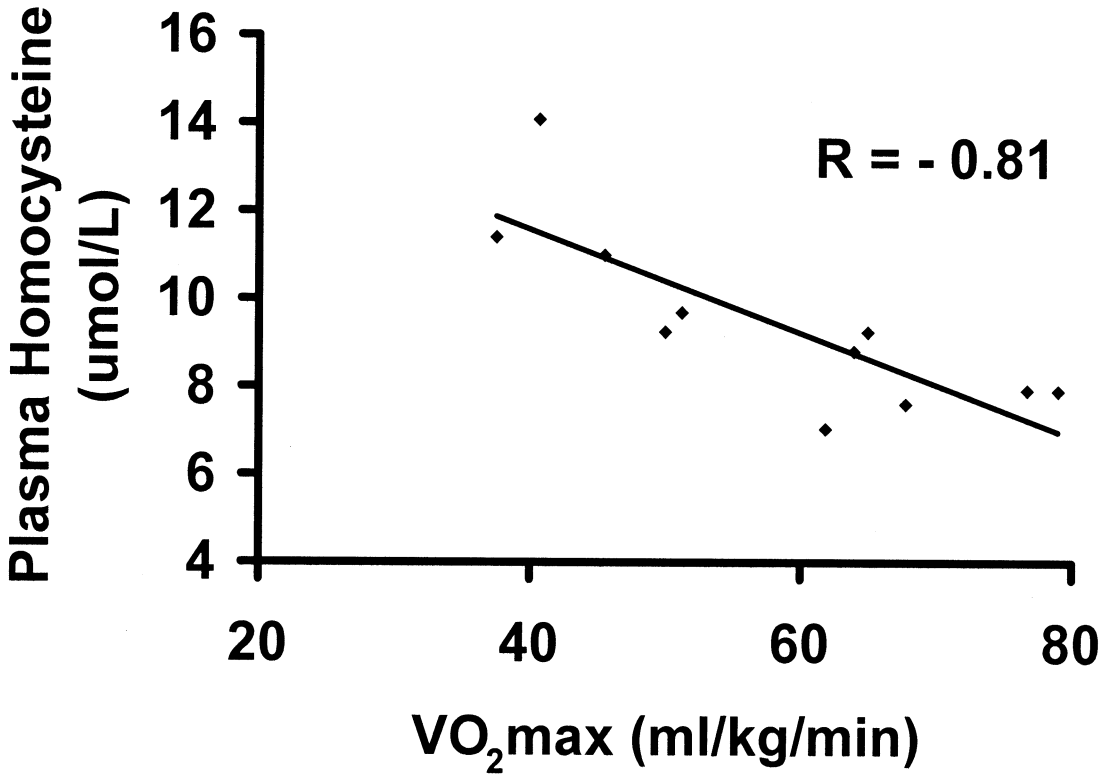

Fig. 1. Relationship between plasma homocysteine and cardiorespiratory fitness in female subjects.

To investigate further the relationships between likely predictors and plasma homocysteine, these variables were divided into percentile-based tertiles; data from these analyses are shown in Table 3. Significant differences ($P < 0.05$) among the tertiles were found only in regard to vitamin B intakes, with differences between each group with each vitamin.

4. Discussion

This is the first study to describe the relationship between measurements of cardiorespiratory fitness and plasma homocysteine. Our cross-sectional data show that cardiorespiratory fitness and plasma homocysteine are significantly negatively related in women but not in men. This supports previous data indicating a sex-related difference in homocysteine metabolism, and may have important implications for the management of hyperhomocysteinemia in women.

The present findings are in contradiction to previous studies that reported significant negative relationships between homocysteine and self-reported physical activity in both men and women [2], in men only [4], a weak positive association in women [5], and no association after adjusting for vitamin B intake [3]. A possible explanation for the divergent

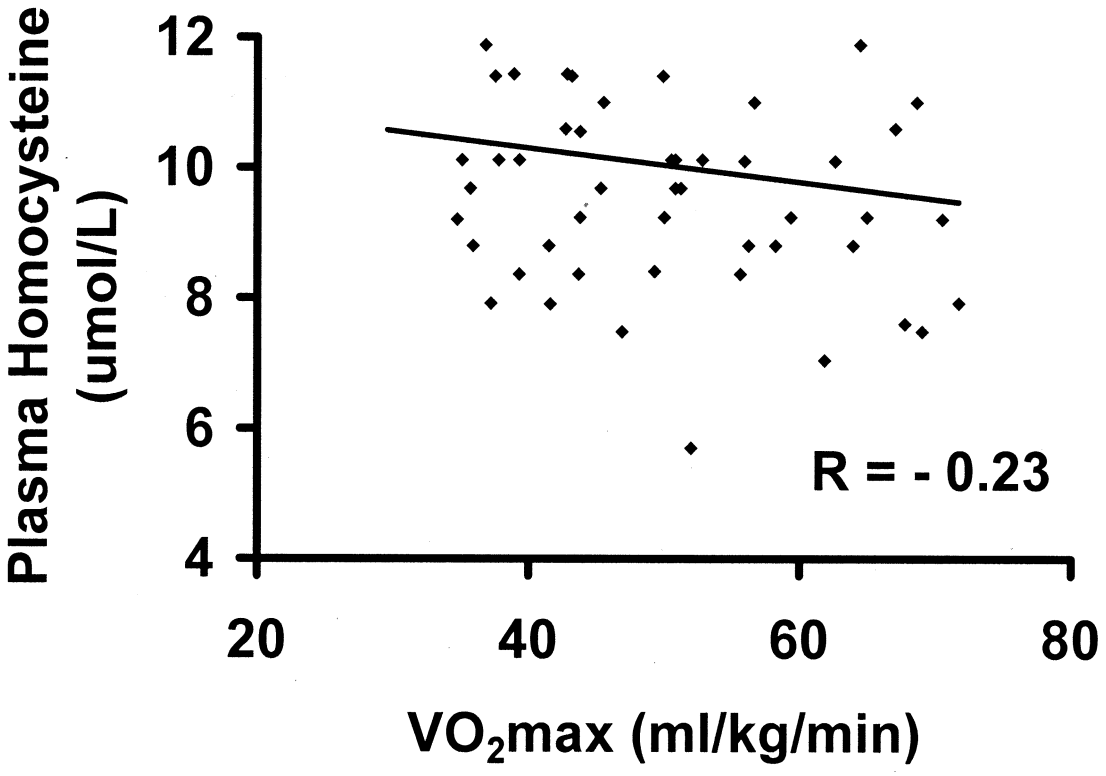

Fig. 2. Relationship between plasma homocysteine and cardiorespiratory fitness in all subjects.

findings is the choice of the tools used to assess physical activity levels in these previous studies. In the present study we objectively measured cardiorespiratory fitness using a validated, widely used and accepted laboratory technique. This is in contrast to the four above-mentioned studies that used self-reporting questionnaires.

The limitations of using questionnaires to assess physical activity levels are well known; they include a lack of standardized constructs, lack of standardized energy expenditure units, a poor understanding of the process associated with recalling physical activity, and substantial inter- and intra-individual variation [6–8].

Sex differences in plasma homocysteine have previously been reported [16]. It is recognized that plasma homocysteine is higher in men than in women, with differences in fat-free mass (FFM) and estradiol concentrations seen as possible explanatory factors [16]. Specifically, homocysteine correlated significantly with FFM and inversely with estradiol. The relationship between homocysteine and FFM was ascribed to the increase in creatinine that was seen in individuals with higher FFM and to the fact that creatinine shares the same metabolic pathway as homocysteine. Thus, an increase in creatinine may result in decreased homocysteine metabolism.

It is difficult to postulate potential mechanisms that may explain the differences in the relationship between cardiorespiratory fitness and homocysteine in women and men. It is known that major cardiovascular and respiratory adaptations that accompany increases in

Table 3
Homocysteine levels by cardiorespiratory fitness, age, body mass index (BMI), and body fat

Variable	*n*	Mean ± SD	*P* (1-Way ANOVA)
Cardiorespiratory fitness (mL/kg/min)			
< 44	19	10.5 ± 1.8	
44–56	21	9.6 ± 2.4	
> 56	20	9.0 ± 2.5	0.14
Age (y)			
< 25	20	9.1 ± 2.9	
25–30	21	9.9 ± 1.7	
> 30	19	10.3 ± 2.1	0.28
BMI			
< 22	20	9.0 ± 2.4	
22–25	20	10.9 ± 2.1	
> 25	20	9.3 ± 2.4	0.06
Body fat (%)			
< 10	20	10 ± 2.5	
10–18	20	9.2 ± 2.5	
> 18	20	10.2 ± 2.1	0.36
Total cholesterol (mmol/L)			
< 4.2	20	9.2 ± 3.0	
4.2–5.0	20	10.7 ± 2.0	
> 5.0	20	9.4 ± 2.0	0.18
LDL cholesterol (mmol/L)			
< 2.2	20	10 ± 2.6	
2.2–3.3	20	9.3 ± 2.6	
> 3.3	20	10.0 ± 1.8	0.41
HDL cholesterol (mmol/L)			
<1.25	20	9.0 ± 2.7	
1.25–1.50	20	10.0 ± 1.6	
>1.50	20	10.4 ± 2.6	0.22
Folate intake (μg)			
<298	20	232 ± 46	
298–411	20	352 ± 29	
>411	20	636 ± 219	<0.001
Vitamin B_{12} intake (μg)			
<1.25	20	2.5 ± 0.6	
1.25–1.50	20	4.3 ± 0.7	
>1.50	20	7.3 ± 1.3	<0.001
Vitamin B_6 intake (mg)			
<1.9	20	1.5 ± 0.3	
1.9–2.5	20	2.2 ± 0.2	
>2.5	20	4.0 ± 1.2	<0.001

physical activity are not sex specific. Furthermore, metabolic and biochemical responses to exercise training are also recognized as being similar between men and women. Given the paucity of research examining the relationships between cardiorespiratory fitness and homocysteine it is not surprising that there is also limited work quantifying changes in the components of metabolic pathways that may explain sex differences in the relationship between homocysteine and cardiorespiratory fitness. These components would include the B vitamins, creatinine, and key enzymes involved in homocysteine metabolism such as cys-

tathionine-β-synthase, and methylenete trahydrafolate reductase (MTHFR). Given the importance of homocysteine in cardiovascular health, these postulates provide an interesting area for future research.

Several limitations of the study may affect the interpretation of the data presented. The number of subjects involved in the project was limited because of the time constraints involved in carrying out objective assessments of cardiorespiratory fitness in the laboratory. Furthermore, the study was not originally designed to determine sex differences, therefore; the inclusion of only 11 women compared to 49 men also represents a significant limitation to the interpretation of the data. Surprisingly, the women who volunteered for the study had a higher VO_2max than the men. We suspect that this was due to self-selection bias, with women with a higher cardiorespiratory fitness being more likely to volunteer. The menstrual status of the women was also not assessed, and it is recognized that there is a close relationship between estradiol and homocysteine status [16]. In addition, although diet diaries were used to assess dietary vitamin B intake, the measurement of plasma B vitamins and other circulating compounds known to be related to homocysteine (such as creatinine) were not determined. Finally, it is well recognised that the MTHFR genotype is a major determinant of plasma homocysteine [17]. Unfortunately, measurement of the MTHFR polymorphism was beyond the financial resources for this project. Given these limitations, the study data provide the first description of the association between homocysteine and cardiorespiratory fitness and warrant further investigation of this relationship.

In conclusion, the present data indicate that there is no observable relationship between cardiorespiratory fitness and plasma homocysteine in men, and a significant inverse relationship in women.

Acknowledgments

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