

Cardiorespiratory Fitness and Plasma Homocysteine Levels in Adult Males and Females

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ABSTRACT: **Background:** High levels of plasma homocysteine constitute a risk for cardiovascular disease. Physical activity, known to reduce CVD risk, has been related to levels of Hcy. Recently, higher Hcy was shown to be associated with lower cardiovascular fitness in women but not in men.

Objectives: To further explore the relationship between cardiorespiratory fitness and plasma total homocysteine levels in a large cohort of adult males and females.

Methods: This cross-sectional study included 2576 fitness and Hcy examinations in adults (62% males) aged 30–59 years randomly drawn from a population undergoing a periodic health examination in the Sheba Medical Center's Executive Screening Survey. Blood tests were collected for tHcy and a sub-maximal exercise test was performed to estimate cardiorespiratory fitness. Information on CVD/CVD risk factors (coronary heart disease, cerebrovascular accident, diabetes, hypertension or dyslipidemia) was self-reported.

Results: Mean tHcy plasma levels were 14.4 ± 7.7 and 10.2 ± 3.0 $\mu\text{mol/ml}$, and mean maximal oxygen uptake 36.5 ± 11.7 and 29.2 ± 9.5 ml/kg/min for males and females, respectively. A multiple regression analysis, adjusting for age, body mass index and CVD/CVD risk factors, showed no association between cardiorespiratory fitness and level of tHcy in males ($P = 0.09$) or in females ($P = 0.62$).

Conclusions: In this sample no association was found between level of cardiorespiratory fitness and plasma tHcy in men or women. The inconsistency of findings and the small number of studies warrant further research of the association between cardiorespiratory fitness and tHcy, an association that may have clinical implications for the modifications of cardiovascular risk factors. *IMAJ 2009;11:78–82*

KEY WORDS: sub-maximal exercise test, maximal oxygen uptake, risk factor, cardiovascular diseases, total homocysteine

Epidemiological studies conducted over the past 25 years have provided ample support for the association of mild hyperhomocysteinemia with an elevated risk of atherothrombosis [1]; however, its causality has not yet been unequivocally confirmed.

Recently, three clinical trials [2–4] reported that reducing the level of homocysteine by administering folic acid and vitamin B12 did not result in any clinical benefit, such as lower cardiovascular events or improved cognitive performance. Loscalzo [5] postulated that a change in the cell phenotype following the use of folic acid and vitamin B12 is responsible for promoting the development of plaque due to alteration in the methylation potential in vascular cells. Thus, an alternative way for reducing Hcy concentrations should be explored.

Elevated level of Hcy is related to poor lifestyle habits such as lack of exercise [6,7]. Recently we showed that a sedentary lifestyle was independently associated with levels of total Hcy in an elderly population, controlling for B vitamin intake and smoking [8] as well as for level of B vitamins (B12 and folate acid) in the plasma and to MTHFR C677T mutation [9]. More physical activity as well as cardiorespiratory fitness may be an alternative approach for reducing Hcy levels without adversely affecting the development of the atherogenic plaque.

Kuo et al. [10] found an inverse association between levels of Hcy and estimated maximal oxygen uptake in the NHANES sample in females but not in males. The finding that physical fitness is related to the level of Hcy differently in males and females is surprising and not readily understood, in as much as Kuo and colleague's [10] attempt to explain difference in cardiorespiratory fitness by change in level of Hcy. The biological plausibility for their hypothesis is unclear to us.

The objective of the present study was to examine the association between cardiorespiratory fitness, as indexed by an estimated $\text{VO}_{2\text{max}}$, and level of tHcy in a group of adult males and females undergoing a periodic health examination.

CVD = cardiovascular disease
Hcy = homocysteine
tHcy = total homocysteine

$\text{VO}_{2\text{max}}$ = maximal oxygen uptake (ml/kg/min)

PATIENTS AND METHODS

The Executive Screening Survey at the Sheba Medical Center performs approximately 9000 screening examinations of adult males and females annually. This population is composed mainly of senior executives referred for periodic health screening by their organizations. A sub-maximal exercise test is routinely performed as part of the physical assessment. A total of 9228 individuals were tested for cardiorespiratory fitness level during their annual Executive Screening Survey medical examination between 2001 and 2002. Plasma tHcy was obtained from 10 randomly selected individuals on each day during the study period.

The present study included 1551 males and 897 females for whom 12 hour fasting tHcy plasma level was measured during their visit to the Executive Screening Survey. Inclusion criteria were age between 30 and 59 years and a complete sub-maximal exercise test. Age was limited to 30–59 for two reasons: the number of subjects younger than 30 was small; and secondly, research findings [8,11] indicated that for individuals older than 59 the concentration of plasma Hcy increases with age, and this increase may confound an inverse association between tHcy and level of physical fitness, particularly after the age of 60.

TESTING PROCEDURE

At the periodic checkup the subjects completed a detailed medical questionnaire and underwent a physical examination and blood tests for total cholesterol, high density lipoprotein and low density lipoprotein-cholesterol, glucose and tHcy drawn after a 12 hour fast. Blood tests were performed by the same laboratory at the Sheba Medical Center. tHcy was determined following separation by high performance liquid chromatography [12].

Sub-maximal Bruce treadmill exercise test was performed using the usual protocol of five stages of increasing strain, each lasting 3 minutes. A valid stage is one in which the heart rate does not exceed the target heart rate [target heart rate = $(220 - \text{age}) \times 0.8$], as recommended by the American College of Sports Medicine [13]. $\text{VO}_{2\text{max}}$ was estimated by using measured heart rate response to known levels of exercise workloads, assuming the relationship between heart rate and oxygen consumption is linear during exercise [13].

Participants provided information on their medical history including a known chronic CVD, and risk factors for CVD. In this study 'CVD/CVD risk factors' included any one of the following: coronary heart disease, history of cerebrovascular accident, diabetes, hypertension, dyslipidemia.

Data on smoking habits were available for 46.9% of the males and 36.2% of the females in the sample, as all individuals in the Executive Screening Survey were asked to report any change in smoking behavior since their previous

examination. Thus smoking information was available only for new Survey members and for old members who reported a change in smoking habits. This may have introduced an information bias such that data on current smoking status are available for a relatively small proportion of participants. Therefore, the quality of available data regarding smoking behavior did not permit integrating them into the models in any meaningful manner.

STATISTICAL ANALYSIS

Parametric variables were compared using the two-tailed Student's *t*-test. Correlations were determined using Spearman's test. Categorical comparisons applied the Spearman chi-square and Fisher tests [14]. Since tHcy concentration is not normally distributed, a natural logarithmic transformation was applied. Linear regression was used to study the relationship between physical fitness and tHcy and included the $\text{VO}_{2\text{max}}$ value, age, body mass index, and the presence of CVD/CVD risk factors. All analyses were performed for men and women separately to control for gender differences in all measured parameters.

The institutional review board committee authorized this study. The clinical data were routinely collected in the Executive Screening Survey as part of the periodic checkups and no additional procedures or tests were involved. The study file contained no personal identification of the participants of any type, and since the study involved a large number of subjects making it impossible to identify subjects, the institutional review board committee waived the informed consent procedure for this study.

RESULTS

Table 1 presents the demographic characteristics and anthropometric and biochemical baseline values of the study sample for males and females separately. Males and females differed significantly in all biochemical and anthropometric measures. They did not, however, show a significant difference in lifestyle habits (i.e., smoking and physical activity) or mean age. The prevalence of CVD/CVD risk factors of the study sample is also given in Table 1. These rates correspond to rates in the Executive Screening Survey population at large and to rates in men and women in the equivalent age group (30–60 years) within the general Israeli population [15].

Table 2 summarizes the multiple regression analysis models of the association between cardiorespiratory fitness and level of plasma tHcy for males and females separately. The first model shows that in males there was no significant relationship between cardiorespiratory fitness, as indexed by $\text{VO}_{2\text{max}}$, and plasma level of tHcy, controlling for age, BMI, and the

BMI = body mass index

Table 1. Demographic and clinical characteristics of the study group

Variable	Males (n = 1602)	Females (n = 974)	P*
Age (mean ± SD)	46.5 ± 7.5	46.1 ± 8.0	0.3
BMI, (kg/m ² , mean ± SD)	26.6 ± 3.2	24.5 ± 4.2	< 0.001
Presently smoking** (%)	16.2	16.6	0.9
Reported habitual physical activity (%)	65.6	62.8	0.3
HDL (mg/dl, mean ± SD)	41.6 ± 9.0	54.8 ± 12.6	< 0.001
LDL (mg/dl, mean ± SD)	125.8 ± 29.6	119.9 ± 29.8	< 0.001
Systolic blood pressure (mmHg)	122.1 ± 15.4	112.8 ± 16.6	< 0.001
Diastolic blood pressure (mmHg)	77.6 ± 9.0	73.8 ± 9.0	< 0.001
VO _{2max} (ml/kg × min)	36.5 ± 11.7	29.2 ± 9.5	< 0.001
Total homocysteine (μmol/ml)	14.4 ± 7.7	10.2 ± 3.0	< 0.001
Coronary heart disease (%)	4.8	0.8	< 0.001
Cerebral vascular accident (%)	0.6	0.2	< 0.001
Diabetes (%)	4.3	1.7	< 0.001
Hypertension (%)	14.0	8.0	< 0.001
Dyslipidemia (%)	30.6	13.2	< 0.001

* P value for comparing males and females in the sample

** Information on smoking status is available for 46.9% of males and 36.2% of females – see Patient and Methods section for detailed explanation.

HDL = high density lipoprotein, LDL = low density lipoprotein

Table 2. Multiple regression analysis for the prediction of total plasma homocysteine level

	Predictive variable	Comparison groups	Coefficient (%)	95% confidence limits (%)	P
Males	VO _{2max} ml/(kg × min)	1 unit increments	-0.1	-0.30 0.02	0.09
	Age (yrs)	1 yr increments	-0.3	-0.55 0.02	0.07
	BMI (kg/m ²)	1 unit increments	-0.2	-0.76 0.44	0.60
	CVD/CVD risk factors	Yes vs. No	4.3	0.2 8.4	0.04
Females	VO _{2max} ml/(kg × min)	1 unit increments	0.06	-0.17 0.29	0.62
	Age (yrs)	1 yr increments	0.08	-0.22 0.38	0.61
	BMI (kg/m ²)	1 unit increments	0.01	-0.52 0.54	0.96
	CVD/CVD risk factors	Yes vs. No	1.6	4.2 7.4	0.57

presence of CVD/CVD risk factors. The model shows that for every unit of increase in the VO_{2max} there is a 0.1% decrease in the plasma tHcy levels (95% confidence interval -0.32%, 0.02%; $P = 0.09$). Similarly, there was no association found between cardiorespiratory fitness and level of tHcy in women ($P = 0.62$). In both males and females the model shows a positive association between the presence of CVD or CVD risk factors and plasma tHcy levels, which was statistically significant in males only. tHcy plasma levels of men with CVD or CVD risk factors were 4.3% higher on average than those of the men free of those conditions (95%CI, 0.2%, 8.4%; $P = 0.04$).

CI = confidence interval

DISCUSSION

This study found no significant relationship between cardiorespiratory fitness and level of plasma tHcy in males or females. Linear regression analyses indicated that in both males and females there was no significant difference in plasma tHcy as a function of cardiorespiratory fitness level, as indexed by VO_{2max} calculated from the time spent in a sub-maximal exercise test. Ruiz et al. [16] in a cross-sectional study of 680 Swedish children and adolescents (9–16 years old) found that tHcy levels were not associated with body fat or any measure of level and pattern of physical activity, or with data on cardiorespiratory fitness, after controlling for potential confounders including MTHFR 677C>T. The finding that tHcy level is unrelated to cardiorespiratory fitness disagrees with previous research on the relationship between physical activity and level of Hcy. König et al. [12], for example, found lower tHcy levels in athletes classified as a high-training group compared to those classified as a low-training group, following a 30 day training period. In the study by Dankner and colleagues [8], physical activity was independently associated with lower level of plasma tHcy in an elderly population, controlling for B vitamin intake and smoking as well as for levels of B vitamins in the plasma and MTHFR 677C>T mutation [9]. Cardiovascular fitness was significantly associated with homocysteine levels in 80 female adolescents, after controlling for potential confounders including the MTHFR 677C>T genotype, but not in 76 male adolescents studied cross-sectionally in Spain [17]. Studies have shown that exercise behavior is a good predictor of cardiorespiratory fitness [1,18].

Following the recently reported clinical trials [2-4] that showed no clinical benefits for reducing Hcy level by administering folic acid and vitamin B12, in terms of lower cardiovascular events or improved cognitive performance, we proposed an alternative way that has the potential to improve tHcy concentrations with a parallel reduction in cardiovascular events by increasing cardiorespiratory fitness.

Our finding that the level of tHcy is not associated with cardiorespiratory fitness is contrary to the study of Kou et al. [10], who found an association between Hcy and cardiorespiratory fitness in women but not in men. The differential association of fitness and tHcy level may suggest that males and females do not benefit in the same way from improved cardiorespiratory fitness in terms of reduced tHcy. Kuo and co-workers [10] speculated that such a difference may be attributed to the differential effect of sex hormones but they could not provide an explanation for the gender-specific effect of cardiorespiratory fitness on Hcy. This is even more puzzling in the light of epidemiological data [19] that show a health benefit for physical activity in both men and women. Both our study and that of Kuo et al. [10] employed large

samples (2448 and 1444; 36.6% and 50.5% females, respectively), minimizing the possibility of lack of statistical power. Further data are needed to establish the gender-mediated relationship between cardiorespiratory fitness and tHcy level, and the potential mechanisms, which could account for such differential association.

In our analysis, however, we controlled for potential covariates that may confound the measured association between cardiorespiratory fitness and plasma tHcy to a limited extent. The presented models controlled for age, BMI and the presence of CVD/CVD risk factor (coronary heart disease, cerebrovascular accident, diabetes, hypertension, hyperlipidemia), which are known to co-vary in relation to Hcy [20] or cardiorespiratory fitness [21] but did not control for health-related behaviors such as smoking habits and nutritional factors. In our study, the limited data on smoking habits did not permit integrating smoking behavior into the models in any meaningful manner. The present proportion of current smokers (16.2% and 16.6% for males and females, respectively) is probably not representative of their true proportion in the sample. Nevertheless, in light of the lack of association between tHcy and cardiorespiratory fitness reported here, adjusting for these factors would have probably further reinforced our findings. In addition, given the inconsistent relationship between smoking and Hcy in the literature [8,22,23], and our concerns regarding reliability of the smoking data, we decided to exclude this factor from the analysis.

Furthermore, one might argue that using a sub-maximal exercise test to infer fitness is less accurate for calculating VO_2 max as it provides an estimated VO_2 max value. An alternative method would be to measure cardiorespiratory fitness via a direct measure of maximal oxygen uptake during the exercise tolerance test. Nevertheless, sub-maximal exercise capacity is a valid measure of cardiorespiratory fitness [24,25] and is often used in the clinical context [10] to measure cardiorespiratory fitness.

We are aware that our study group, much like the entire population of the Executive Survey, is not representative of the general Israeli adult population and comprises a relatively high socioeconomic group; most individuals were employed at the time of the examination, and the rate of smoking was low compared to the general population. In addition, women are slightly under-represented in the study (38% of the sample). Nevertheless, the prevalence rate of CVD and cardiovascular risk factors besides smoking rates are similar to those reported by the Israel Center for Disease Control [15].

Our finding of an inverse association between physical activity and tHcy [8,9] and the reported findings of elevated levels of Hcy as independently associated with low cardiovascular fitness in women [10] and girls [17] but

not in men or boys led us to further examine the tHcy-fitness relationship in our sample. However, we found no association between cardiorespiratory fitness and tHcy level in adult men and women, a finding consistent with the negative association reported in children and adolescents [16]. It could be that there is no association between cardiorespiratory fitness and level of plasma tHcy or it may be that methodological weaknesses have masked a relationship otherwise apparent. Further studies are needed to elucidate this clinically relevant paradigm.

In conclusion, the present study found no relationship between level of cardiorespiratory fitness and plasma tHcy in men or in women. The inconsistent data in the literature and the small number of studies conducted on this subject warrant further research of the association between cardiorespiratory fitness and tHcy, an association that may have clinical implications for the modification of cardiovascular risk factors. However, our findings may also suggest that further attempts to shed light on the independent association between sedentary lifestyle and tHcy should consider alternative target systems to the cardiorespiratory system.

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