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RELATIONSHIP OF PLASMA HOMOCYSTEINE LEVELS TO PHYSICAL ACTIVITY AND AEROBIC FITNESS IN YOUNG MEN

J.L.P.Roy[1], M.T.Richardson[2], J.F.Smith[3], Y.Neggers[4], R.Lomax[5], R.Pieroni[6], G.R.Hunter[7]

[1]Dept. of Human Studies, University of Alabama at Birmingham, Birmingham; [2]Dept. of Kinesiology, The University of Alabama, Tuscaloosa; [3]Dept. of Kinesiology, The University of Alabama, Tuscaloosa; [4]Dept. of Human Nutrition and Hospitality Management, The University of Alabama, Tuscaloosa; [5]Dept. of Educational Research, The University of Alabama, Tuscaloosa; [6]Dept. of Internal Medicine, The University of Alabama, Tuscaloosa; [7]Dept. of Human Studies, University of Alabama at Birmingham, Birmingham, USA

Abstract. Recent evidence has suggested elevated plasma Homocysteine (Hcy) level is an independent risk factor for vascular diseases. Although its association with other established risk factors for cardiovascular disease (CVD) has been studied, there is inconclusive evidence regarding the relationship between aerobic fitness (AF)/physical activity (PA) levels and plasma Hcy. The purpose of this study was to examine the relationship between aerobic fitness/PA and plasma Hcy level. Subjects included 30 healthy males aged 20-35 years who were non-smokers, not diabetic, and ate a diet that was not deficient in folic acid. Subjects performed a Bruce VO_{2max} test to assess AF; answered the comprehensive Stanford Seven-Day Recall questionnaire (Stanford 7-DR) to assess total physical activity (S-PA), very hard physical activity (S-VPA), and hard and very hard physical activity (S-HVPA), and a single-item, four-level PA questionnaire to assess global PA (G-PA). A fasting blood sample was drawn and analyzed for plasma Hcy, triglycerides (Trig), total cholesterol, high density lipoprotein cholesterol (HDL-C), and low density lipoprotein cholesterol (LDL-C) levels. The following correlation coefficients between plasma Hcy level and VO_{2max}, G-PA, S-PA, S-HVPA and S-VPA were not statistically significant (i.e. r values ranged from -0.05 to -0.18, $P>0.05$). This study did not demonstrate a significant relationship between plasma Hcy and AF or PA assessed by the Stanford 7-DR. However, the relationship between Hcy and G-PA approached statistical significance ($r=-0.32$, $P=0.08$). There were 5 subjects who were classified as having hyper Hcy (mean Hcy level = 26.1±8.96 μmol/L) and 25 who were within the normal range (mean Hcy level = 8.0±1.46 μmol/L). T-tests revealed no

Reprint request to: Dr. Jane Roy, EB 232N, 1530 3rd Ave S, Birmingham, AL 35294; Tel.: (H) 205-981-3373, (W) 205-934-1757; Fax: (W) 205-975-8040; E-mail: jroy@uab.edu

significant differences between the hyper Hcy and normal Hcy groups, except for percent body fat (%BF) (P=0.023). In conclusion, plasma Hcy level was not associated with AF/PA, with the possible exception of G-PA and %BF.

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Key words: Homocysteine - Aerobic fitness - Physical activity - Folic acid - Cardiovascular disease

Introduction

Cardiovascular disease (CVD) is the number one cause of premature death in the United States. Established modifiable risk factors associated with CVD include high cholesterol, smoking, hypertension, obesity and physical inactivity/low fitness [6,25,44,68]. There is a current interest in Hcy because evidence has suggested hyper Hcy is an independent risk factor for vascular diseases [28,46,64,70]. The determinants of plasma Hcy concentration include nutritional (e.g. dietary intake of folic acid, [22,33,42], genetic [13,21,29] and endocrine (e.g. diabetes mellitus) [35] factors. Elevated homocysteine levels are referred to as hyperhomocysteinemia (hyper Hcy), and several recent studies have indicated a strong association between mild to moderate hyper Hcy and vascular diseases [5,11,14,17,29,30,43,53,55,57, 62,65,67]. Several mechanisms regarding the possible atherogenic effects of hyper Hcy have been proposed [34,45,47,50].

Improving aerobic fitness (AF) and physical activity (PA) levels is being promoted more vigorously now than ever to decrease the risk of CVD [25]. Whereas the favorable alterations on blood levels of high density lipoproteins (HDL-C) and triglycerides (Trig), blood pressure, and obesity with regard to AF/PA are well known [3,8,69], the relationship between plasma Hcy and AF/PA remains inconclusive [23,49,52,53,62,72,75]. The relationship between plasma Hcy and other established risk factors for CVD has been studied extensively in both CVD patients and controls, yet the associations are not clear. A lack of association has been demonstrated between plasma Hcy and total cholesterol (total-C) and low density lipoprotein (LDL-C) levels [14,29,37,55,67], blood pressure [20,37,55], body composition [55,67], smoking [20], diabetes [20], Trig [5,14,29], age [29,53, 55], and HDL-C [5,29,55,67). Conversely, significant positive associations between plasma Hcy and total-C and LDL-C [5,37,52], blood pressure [5,29,37,41, 49,52,67], smoking [5,7,23,52,61], diabetes [35], age [30,37,41,52,61,67], and male gender [30,52,61] have been demonstrated in several studies. A significant

inverse relationship has also been demonstrated between plasma Hcy and HDL-C levels [30]. Possible reasons for these discrepancies include varying study methodologies, subject selection criteria, data analysis and Hcy level categorizations.

Two studies found a significant relationship between PA and plasma Hcy levels. Nygard *et al.* [52] conducted a large-scale epidemiological study involving 7591 men and 8585 females, aged 40-67 years. PA was evaluated by a global self-reported questionnaire. Subjects were asked to choose one of four categories that best fit their average degree of activity for the last year. It was concluded that elevated plasma Hcy level was associated with an inactive lifestyle. Mennen *et al.* [49] evaluated the determinants of Hcy in healthy French adults using a global PA questionnaire that categorized PA into 3 classes and found that Hcy was inversely related to PA in men only (P=0.04).

Four studies found no significant relationship between PA and plasma Hcy levels. Pancharuniti *et al.* [53] conducted a case control study (101 white males aged 30-50 years with angiographically demonstrated CAD, and 108 white male similarly aged control subjects). PA was rated by asking participants to describe their PA level during work and leisure as little or none, occasional, or regular (three or more times a week). This study did not demonstrate a significant correlation between PA and Hcy levels. De Bree *et al.* [23] investigated the extent to which PA was associated with plasma Hcy level in a large scale epidemiological study (n=1493 men and n=1532 women). PA was reported as the average amount of leisure time (minutes/week) spent in various activities over the past year. For the statistical analysis PA was split into four categories ranging from sedentary to very active. The authors concluded that Hcy level was not strongly associated with PA. Volek *et al.* [72] investigated the influence of an 8-week weight loss program that included diet and exercise modification components. They concluded that short term weight loss resulting from reducing percentage energy intake, increasing PA and vitamin/mineral supplementation had a favorable effect on regional body composition with a minimal effect on Hcy. Saw *et al.* [61] investigated the relationship between plasma Hcy and PA levels in middle age and older men and women. PA was rated into two categories; having more or less than 0.5 hours/week in activities such as jogging or brisk walking. It was concluded that plasma Hcy concentration was not associated with PA after adjustment for plasma folate concentration.

Wright and Francis [75] investigated the relationship between plasma Hcy and aerobic fitness. No significant correlation was found between AF and plasma Hcy levels in a sample of 20 relatively highly fit men (r= -0.05).

The purpose of this study was to examine the relationship between plasma Hcy and AF/PA levels in thirty young males with a range of AF/PA levels. The hypothesis underlying this study is that subjects with higher levels of AF/PA have lower plasma Hcy levels, and therefore less risk of developing CVD. The present study attempted to extend previous work by assessing PA with the comprehensive Stanford Seven-Day Recall questionnaire (Stanford 7-DR) [58] in addition to the single-item global questionnaire developed by Nygard *et al.* [52], and by studying subjects of various fitness levels ranging from low to highly fit. In addition, the relationship between plasma Hcy and other CVD risk factors (e.g. percent body fat (%BF), body mass index (BMI), total-C, HDL-C, LDL-C and Trig levels) was examined.

Material and Methods

Approval for the study was granted by The University of Alabama Institutional Review Board for research with human subjects. All subjects signed an informed consent and answered a medical history questionnaire to screen for contraindications to performing a treadmill VO_{2max} test [2] before participating in this study. Subjects were 30 caucasian male volunteers, aged 20-35 years, with no known health problems. Non-caucasian males were excluded from the study as there is evidence of ethnic related plasma Hcy differences [70]. Criteria for exclusion included:

a) a diet that was deficient in folic acid assessed by a comprehensive food frequency questionnaire (FFQ) [10]. (Data was collected when the RDA was 200μg [27], but data analysis occurred after the RDA was updated to the current RDI of 400μg in 2001. At the time of data collection we excluded subjects who had less than 2/3 of the RDA (<133μg), which was considered a safe, adequate dietary requirement among people. Data analysis occurred after the guidelines were updated [26], and now six subjects had less than 2/3 of the current RDI (<267μg). However, this did not effect reported results because plasma Hcy was not related to dietary folic acid intake, r=-0.18, P=0.33, which indicated that the subjects were a relatively homogeneous population regarding folic acid ingestion. Furthermore, a t-test revealed no significant difference (P=0.36) in daily dietary folic acid intake between the six subjects that ingested less than 267 μg per of folic acid and the remaining twenty four subjects);

b) smoking status (which was identified as having smoked more than 10 cigarettes during the last 12 months and more than 100 cigarettes during one's lifetime);

c) diabetes mellitus;

d) high alcohol intake (on average, more than one drink per day);

e) drug/medication use that may have an effect on plasma Hcy levels (e.g. NSAIDs, antiseizure medications and nitrous oxide anesthesia);

f) a known family history of elevated Hcy;

g) any contraindications to maximal exercise testing.

The most accurate method of analyzing folic acid status is via red blood cell analysis. Due to financial limitations we assessed folic acid intake by a FFQ [10] that provided a measure of "usual" intake during the past year. The data were analyzed by a Diet QL Version 2.1 computer program (National Cancer Institute, National Institute of Health), which calculated the average daily nutrient values (including folic acid) for each subject. This method of dietary recall is more useful in determining the relationship of nutrition to chronic disease states than is dietary assessment during a shorter period of time, such as a specific day or week (60). Subjects also answered a non-exercise self-report aerobic fitness prediction questionnaire in an attempt to stratify subjects ranging from low to high fit [1].

Age (years), height (m), weight (kg), body composition (%BF), and resting blood pressure (mmHg) [18] were measured and recorded. Height and weight were measured using a physician's scale with the subject dressed in shorts and without shoes. BMI was calculated by dividing the subject's weight (kg) by the square of his height (m^2) [56]. Body composition was determined by skinfold measurement using Lange calipers (Cambridge Scientific Industries, Inc., Cambridge, England). %BF was estimated from the sum of chest, abdomen, and thigh skinfold thicknesses using the table compiled by Pollock *et al.* [56].

PA was assessed using the comprehensive Stanford 7-DR questionnaire [58]. Subjects were asked to recall the number of hours spent sleeping and engaged in moderate, hard, and very hard physical activity during the previous seven days. Metabolic equivalent levels (METS) were assigned to each class of activity (i.e. sleep = 1 MET; light = 1.5 METS; moderate = 4 METS; hard = 6 METS, and; very hard = 10 METS) and results were expressed in kcal/kg/day. Subjects also answered a global PA (G-PA) assessment questionnaire as described by Nygard *et al.* [52] in which subjects were asked to mark one of four categories that best described their average level of leisure time PA for the last year.

Within one week of the blood draw, aerobic fitness was measured by a graded Bruce VO_{2max} (ml/kg/min) test [15] which took place on a Quinton Q55XT motorized treadmill (Quinton Instrument Company, Seattle, WA), with heart rate monitored by a Polar monitor (Polar, Port Washington, NY). Rating of Perceived Exertion (RPE) was monitored using the Borg scale [12]. The respiratory measures

(oxygen uptake [O_2], volume of expired air [VE], and respiratory exchange ratio [RER]) were measured using an Aerosport TEEM 100 Metabolic Analyzer (Aerosport, Ann Arbor, MI) that was calibrated with standardized gases prior to each measurement. The Aerosport TEEM 100 Metabolic Analyzer has been shown to be a reliable and valid measurement of VO_{2max} [48,51,73]. Expired gases were collected by a Hans-Rudolph model 8900 face mask that was secured by a Hans Rudolph adult series head cap (Hans-Rudolph Inc., Kansas City, MO). Test end-points included subject volitional fatigue and/or any signs or symptoms of exercise intolerance. The test was considered a maximal test if two out of the four American College of Sports Medicine (ACSM) criteria were met [3]. Subjects who did not reach the criteria for VO_{2max} were not included in the results.

Subjects arrived for the blood draw in the morning after a 12-14 h fast, and were instructed not to perform any strenuous exercise prior to their visit. After reaching the laboratory, subjects rested for 20 min before 10 ml of blood was drawn from each subject's brachial vein by a trained phlebotomist. Whole blood was collected in a serum separator tube for the lipid analysis that was allowed to clot for 10 minutes before being centrifuged for 10 min at 3400 rpm. Total cholesterol was analyzed based on the enzymatic method similar to that proposed by Allain *et al.* [2]; HDL-C was analyzed using a magnetically enhanced reagent containing dextran sulfate and magnesium choloride [74]; LDL-C was assessed using Labdaq 2000 computer software (Ntech Inc., Baltimore, MD); and Trig level was analyzed based on an enzymatic method described by Spayd *et al.* [65]. Whole blood was collected in a EDTA anticoagulant tube and centrifuged immediately at 3400 rpm for 10 min, then immediately removed from the cells and kept frozen at -20°C and analyzed for Hcy level following the protocol described by Cornwell, Morgan, and Vaughn [19]. Each sample was ran twice and the average of the two values was recorded.

The data were analyzed using Pearson correlation coefficients (r) (except for analyses involving G-PA in which Spearman correlation coefficients (rho) were utilized) to determine the relationships between measures of aerobic fitness/PA and plasma Hcy, lipids (Trig, total-C, HDL-C, LDL-C), BMI, and %BF. Correlation coefficients were also utilized to determine the relationships among measures of plasma Hcy, lipids (Trig, total-C, HDL-C, and LDL-C), BMI, and %BF. T-tests comparing mean values of AF, PA, lipids, BMI and %BF between those individuals having hyper and normal Hcy values were run.

As a measure of reliability for the plasma Hcy analysis, an intraclass correlation ($R_{xx'}$) procedure was performed between the first and second measures. To see if dietary intake of folic acid played a role in plasma Hcy levels, a Pearson

correlation (r) analysis was performed between average folic acid intake and plasma Hcy level. Statistical significance for all testing was set at the $P<0.05$ level.

Results

Table 1
Anthropometric, aerobic fitness/physical activity, plasma Hcy and lipid measurements (mean ±SD) of subjects (n=30 males)

Characteristic	Mean ±SD	Range
Age (yr)	25.3±4.0	20-35
BMI (kg/m^2)	26.7±4.5	19.8-35.9
%BF	15.2±5.9	7.0-23.8
VO_{2max} (ml/kg/min)	49.1±13.0	28.0-80.3
S-PA (kcal/kg/day)	36.8±4.4	31.5-45.8
S-HVPA (kcal/kg/day)	3.7±4.0	0.0-15.4
S-VPA (kcal/kg/day)	2.8±2.8	0.0-10.0
G-PA (ordinal units)	2.6±1.2	1.0-4.0
Hcy (μmol/L)	11.1±7.7	5.2-37.9
Trig (mg/dl)	113.7±63.6	38.0-284.0
Total-C (mg/dl)	185.1±31.1	133.0-258.0
HDL-C (mg/dl)	45.2±9.9	27.0-67.0
LDL-C (mg/dl)	120.1±26.0	76.0-169.0
Total-C/HDL-C ratio	4.3±1.2	2.4-7.1

S-PA, S-HVPA and S-VPA = total PA, hard and very hard PA, and very hard PA levels, respectively, as measured by the Stanford 7-DR questionnaire [59];
G-PA = level of PA as measured by the single-item, four-level global PA questionnaire [52];
BMI = body mass index; %BF = percent body fat (via skinfold measurements); Hcy = plasma homocysteine; Trig + triglycerides;
Total-C = total cholesterol; HDL-C = high density lipoprotein choleaterol;
LDL-C = low density lipoprotein cholesterol; Total-C/HDL-C ratio = CHD risk assessment

Note: folic acid mean ±SD = 450±260 μg/day, range = 190-1455 μg/day

Anthropometric, Aerobic Fitness/PA, Plasma Hcy and lipid measurements are presented in Table 1. The frequencies for G-PA levels were as follows: category 1 (sedentary or no activity), n=7; category 2 (walking, cycling, or other type of moderate physical activity for at least 4 hours a week), n=4; category 3 (exercise, gardening with physical exertion, or similar degree of physical activity for at least 4 hours a week), n=9; and category 4 (regular heavy training or competitive sport several times a week), n=10.

Table 2
Correlation coefficients** between measures of aerobic fitness/physical activity and Hcy, lipids, BMI, and BF (n=30 males)

Measure	VO_{2max} (ml/kg/min)	S-PA (kcal/kg/day)	S-HVPA (kcal/kg/day)	S-VPA (kcal/kg/day)	G-PA
Hcy (μmol/L)	-0.18	-0.05	-0.08	-0.08	-0.32 (P=0.08)
Trig (mg/dl)	-0.44*	-0.21	-0.21	-0.32	-0.29
Total-C (mg/dl)	-0.36*	-0.24	-0.22	-0.28	-0.30
HDL-C (mg/dl)	0.34 (P=0.06)	-0.07	0.15	0.17	0.10
LDL-C (mg/dl)	-0.36*	-0.16	-0.19	-0.23	-0.26
BMI (kg/m^2)	-0.40*	0.30	0.00	0.13	0.13
%BF	-0.65*	-0.16	-0.35 (P=0.05)	-0.29	-0.28

*Significant at P<0.05;
**The pearson correlation coefficient (r) was used for all correlations except for G-PA, in which the spearman correlation (rho) coefficient was used

Correlation coefficients between measures of AF/PA and plasma Hcy, lipids, and the other established CVD risk factors assessed in this study (lipids, BMI, and %BF) are presented in Table 2. Correlation coefficients among plasma Hcy and other established CVD risk factors (lipids, BMI, and %BF) are presented in

Table 3. Correlation coefficients between measures of folic acid, plasma Hcy, and AF/PA are presented in Table 4. There were no significant correlations between plasma Hcy and any of these measures (P>0.05).

Table 3
Pearson correlation coefficients between measures of plasma Hcy, lipids, BMI and BF (n=30 males)

Measure	Hcy (μmol/L)	Trig (mg/dl)	Total-C (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	BMI (kg/m^2)	BF (%)
Hcy (μmol/L)	---	0.04	-0.10	-0.28	-0.01	0.00	0.26
Trig (mg/dl)		---	0.73 *	-0.37 *	0.58 *	0.21	0.31 (0.09)
Total-C (mg/dl)			---	-0.12	0.89 *	0.04	0.24
HDL-C (mg/dl)				---	-0.41 *	-0.23	-0.41 *
LDL-C (mg/dl)					---	0.05	0.25
BMI (kg/m^2)						---	0.61 *
%BF							---

*Significant at P<0.05

The mean value for plasma Hcy (11.1 μmol/L) was well within the "normal" range (5-15 μmol/L) for young males. However, three subjects were classified as having "moderate hyper Hcy" (16-30 μmol/L), and two subjects were classified as having "intermediate hyper Hcy" (31-100 μmol/L) [40]. Means, standard deviations and t-tests (significant at P<0.05) were performed between the subjects who were classified as having hyper Hcy (n=5; mean Hcy level = 26.1±8.96 μmol/L) and those who were within the normal range (n=25; mean Hcy level = 8.0±1.46 μmol/L) for AF, G-PA, age, BMI, %BF, Total-C, HDL-C, LDL-C. There were no significant differences between the groups except for %BF (P=0.023).

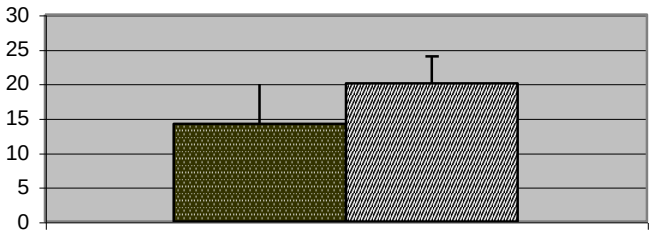
Table 4
Correlation coefficients** between measures of folic acid, plasma Hcy, and aerobic fitness/physical activity levels (n=30 males)

Measure	Folic acid (µg/day)	Hcy (µmol/L)	VO_{2max} (ml/kg/min)	S-PA (kcal/kg/day)	S-HVPA (kcal/kg/day)	S-VPA (kcal/kg/day)	G-PA
Folic acid (µg/day)	---	-0.18	0.76*	0.31 (P=0.09)	0.60*	0.61*	0.42*
Hcy (µmol/L)		---	-0.18	-0.05	-0.08	-0.08	-0.32 (P=0.08)
VO_{2max} (ml/kg/min)			---	0.41*	0.63*	0.65*	0.64*
S-PA (kcal/kg/day)				---	0.82*	0.67*	0.73
S-HVPA (kcal/kg/day)					---	0.84*	0.82*
S-VPA (kcal/kg/day)						---	0.80*
G-PA							---

*Significant at P<0.05
**The pearson correlation coefficient (r) was used for all correlations except for G-PA, in which the spearman correlation (rho) coefficient was used

Further Pearson correlation analysis revealed a significant negative correlation between %BF and dietary folic acid level, r=-0.46, SEE=0.11. The mean and SD's for %BF, G-PA and AF (VO_{2max}) for the hyper Hcy and normal Hcy groups are contained in Fig. 1a, 1b and 1c. An intraclass correlation was ran between the first and second measure of plasma Hcy to determine the reliability of the Hcy assay procedure. The correlation coefficient ($R_{xx'}$) was 0.99 ($P<0.05$), which indicated a high reliability of the test procedure.

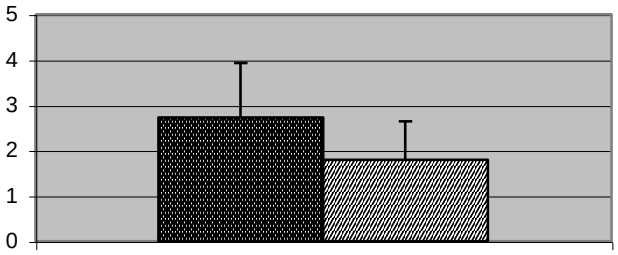
%BF



Normal Hcy Group Hyper Hcy Group

Fig. 1a
The mean comparison of %BF with normal Hcy (n=25) and hyper Hcy (n=5) groups; significant at P=0.023

G-PA



Normal Hcy Group Hyper Hcy Group

Fig. 1b
The mean comparison of G-PA with the normal Hcy (n=25) and hyper Hcy (n=5) groups; No significant difference, P=0.74

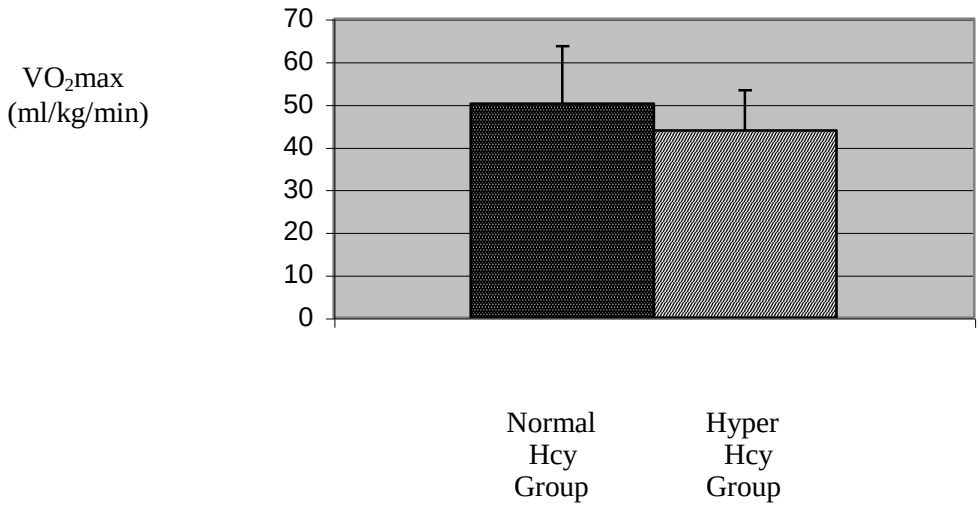


Fig. 1c
The mean comparison of VO_2max with the normal (n=25) and hyper Hcy (n=5) groups; No significant difference, P=0.246

Discussion and Conclusions

This study did not demonstrate a significant relationship between AF and plasma Hcy. These results were in agreement with the only other published study (to our knowledge) that has examined the relationship between plasma Hcy and aerobic fitness [75]. Although the present study attempted to stratify subjects with respect to AF/PA levels, there were still too few low fit, sedentary subjects. To fully explore the dose response relationship between AF/PA and a health outcome (i.e., plasma Hcy level) the full range in AF/PA values should be studied [9,39]. It is possible that a cross-sectional study comparing a larger number of individuals with a greater range in fitness levels (including subjects with very low fitness levels; i.e., below 25 ml/kg/min) might demonstrate a significant difference in plasma Hcy level between "very low fit" versus the "higher fit" groups.

Likewise, there was no significant relationship between plasma Hcy and measures of PA. Our results were consistent with four other studies that found no statistical relationship between PA and plasma Hcy [23,53,62,72]. However, the inverse relationship between the global assessment of PA (G-PA) and plasma Hcy approached statistical significance (r=-0.32, P=0.08). The global assessments of PA (G-PA) used by Nygard *et al.* [52] and Mennen [49] did show significant relationships between plasma Hcy and levels of PA in middle aged and older men.

The present study used only young male subjects and did not have the statistical power to evaluate the significance of differences in plasma Hcy levels of this magnitude (0.76-1.08 μmol/L).

The Stanford 7-DR was chosen as a measure of PA because it appears to be a reasonably valid measure of activity of a week's duration [54]. However, the extent to which activity varies from week to week is unknown, and although none of the subjects reported an atypical week, the 7 days' worth of PA data may not have been representative of habitual activity level. The less detailed global G-PA questionnaire may have been more representative of habitual PA level and, thus, was more strongly related to plasma Hcy than the Stanford 7-DR. Similar findings were reported by Sallis *et al.* [59], where a global measure of PA was more strongly associated with CVD risk factors than the more detailed Stanford 7-DR.

There were no significant correlations (r) between plasma Hcy levels and the established risk factors for CVD evaluated in this study: total-C, LDL-C, HDL-C, Trig, BMI, and %BF. This is consistent with the findings of several other studies which also failed to demonstrate a significant relationship between plasma Hcy and total-C or LDL-C [14,29,37,55,67], HDL-C [5,29,55,67], Trig [5,14,29], and %BF levels [55,67]. However, the literature is discordant regarding the relationship between plasma Hcy and these CVD risk factors as several studies have reported significant correlations for total-C or LDL-C [5,37,52] and HDL-C levels [30]. Reasons for these discrepancies remain unclear. However, it should be appreciated that, in general, for the studies that did show significant relationships, the correlations were relatively modest (ranging from r=0.13 to r=-0.24). Although no significant correlations were reported in the present study, the correlation coefficients were comparable to several studies with sample sizes much larger than ours that reported significant associations.

For the present study, it was concluded that AF/PA were not significantly associated with plasma Hcy levels in normal, healthy men, aged 20-35 years. However, the inverse relationship between plasma Hcy and G-PA approached statistical significance (P=0.08), which suggested that similar comparisons in a larger sample with a wider range in aerobic fitness/PA levels may reveal significant associations. Although AF (VO_{2max}) was not significantly related to plasma Hcy level, it was significantly related to other CVD risk factors, and the relationships were in the expected directions. Plasma Hcy was not significantly associated with the other established CVD risk factors investigated in this study (total-C, LDL-C, HDL-C, Trig, BMI, and %BF). However, the correlation coefficients were comparable to other studies that did report statistically significant relationships and used much larger sample sizes.

The prevalence of hyper Hcy in this study of young healthy males was rather high (5/30 = 17%), but the overall mean for the group was comparable with other young males from Alabama [75] and Europe [32]. None of the subjects from the hyper Hcy group (n=5) had a G-PA rating of category 4, which indicated that none of hyper Hcy subjects reported doing regular heavy training or competitive sport several times a week. To investigate the data further, t-tests were ran to see if there were differences between the elevated Hcy subject group (n=5) and the normal Hcy subjects (n=25) for AF, G-PA, age, BMI, %BF, Total-C, HDL-C, LDL-C. There were no significant differences between the groups except for %BF (P=0.023) suggesting that having a low percent body fat may have a protective effect against elevated Hcy levels. Of course differences in eating habits may have been responsible for the differences in both Hcy and %BF, i.e. fatter individuals may have eaten a high fat diet that is low in folic acid. Pearson correlations revealed a significant negative correlation between %BF and folic acid level, r=-0.46, SEE=0.11. These results indicated that the individuals with the lowest %BF levels tended to eat a diet rich in whole grains, vegetables and folic acid. These findings are consistent with the known effect that folic acid has on plasma Hcy level [33,42]. Volek *et al.* [72] investigated the combined effects of diet, exercise, behavior modification and folic acid supplementation on weight loss. Although there were favorable effects on regional body composition, there was a minimal effect on plasma Hcy concentration. Further studies should examine the role that diet, weight loss and increasing levels of PA/AF may play in lowering plasma Hcy levels, which may reduce the risk of CVD.

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