Cardiovascular Fitness Is Negatively Associated With Homocysteine Levels in Female Adolescents

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Objective: To examine the association between cardiovascular fitness and homocysteine levels in adolescents.

Design: Cross-sectional study.

Setting: Madrid, Murcia, Granada, Santander, and Zaragoza, Spain.

Participants: One hundred fifty-six Spanish adolescents (76 boys and 80 girls) aged (mean±SD) 14.8±1.4 years.

Main Exposures: Cardiovascular fitness was measured by the 20-m shuttle run test. Pubertal stage, birth weight, smoking status, and socioeconomic status were determined, and the sum of 6 skinfold thickness measurements, and serum folic acid and vitamin B12 levels were measured. Methylenetetrahydrofolate reductase (MTHFR; 677C>T genotype) polymorphism was done by DNA sequencing.

Main Outcome Measure: Fasting homocysteine levels.

Results: Mean values of homocysteine were significantly higher in the MTHFR 677CT and TT genotype subgroups compared with the CC genotype subgroup in adolescent boys, whereas in adolescent girls, mean values of homocysteine were significantly higher in the MTHFR 677CT and TT genotype subgroup compared with the CC and CT genotype subgroups. Multiple regression analyses showed that cardiovascular fitness was significantly associated with homocysteine levels in female adolescents after controlling for potential confounders including the MTHFR 677C>T genotype (β = −0.40; semipartial correlation = −0.35; P = .007). No associations were found between cardiovascular fitness and homocysteine levels in male adolescents (β = 0.12; semipartial correlation = 0.08; P = .51).

Conclusion: The results suggest that cardiovascular fitness is negatively associated with homocysteine levels in female adolescents after controlling for potential confounders including MTHFR 677C>T genotype.

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HOMOCYSTEINE HAS BEEN suggested to be an independent risk factor for several multisystem diseases, including coronary heart disease, stroke, dementia, and Alzheimer disease, as well as for risk of hip fracture and pregnancy complications. Moreover, elevated homocysteine levels have been associated with increased oxidative stress and endothelial damage, although the mechanisms are not yet clarified. In children, elevated homocysteine levels are positively associated with cardiovascular disease in their parents, grandparents, and other relatives. Homocysteine levels are influenced by modifiable and nonmodifiable factors. Among the nonmodifiable factors, age and sex seem to have a specific role. Levels of homocysteine are higher in adolescent boys than in adolescent girls, and this sex effect seems to be enhanced during and after puberty. Genetic factors also seem to affect homocysteine levels. Elevated levels of homocysteine can be caused by mutations in enzymes involved in homocysteine metabolism, which give dysfunctional enzymes, for example, the single-nucleotide polymorphism at position 677 in the methylenetetrahydrofolate reductase (MTHFR) gene for MTHFR. Methylene tetrahydrofolate reductase is a key enzyme in homocysteine metabolism. The common polymorphism 677C>T gives a thermolabile form of the enzyme. Subjects homozygous for this mutation (or TT genotype) have higher levels of homocysteine compared with subjects with CC or CT genotypes.

Deficient serum levels of both folic acid and vitamin B12 have been associated with elevated homocysteine levels in children, adults, and elderly persons. Lifestyle factors such as smoking, lack of physical activity, excessive alcohol intake, and obesity have been associated with elevated levels of homocysteine in adults.

Poor cardiovascular fitness (CVF) is another important risk factor for cardiovascular disease and is a predictor of morbidity and all-cause mortality. Kuo et al have recently described a significant negative association between CVF and homocysteine levels in women.
Cardiovascular fitness has been negatively associated with features of metabolic syndrome in children and adolescents and with plasma lipid profile in both overweight and nonoverweight adolescents. However, studies examining the association between CVF and homocysteine levels in adolescents are lacking. We hypothesized that there would be a negative correlation between CVF and homocysteine levels in adolescents. For public health strategies and preventive purposes, it is of interest to understand the relative influence of modifiable factors on homocysteine levels from an early age.

METHODS

PARTICIPANTS

The study participants were a subsample of the AVENA (Alimentación y Valoración del Estado Nutricional de los Adolescentes Españoles [Food and Assessment of the Nutritional Status of Spanish Adolescents]) study, which was designed to assess the health and nutritional status of adolescents. The AVENA study design has been reported in detail elsewhere. Data were collected from November 6, 2000, to June 28, 2002, in 5 Spanish cities: Madrid, Murcia, Granada, Santander, and Zaragoza. Data in the present article are from adolescents in whom both homocysteine levels and MTHFR genotypes were measured (n = 156; 76 boys and 80 girls).

A comprehensive verbal description of the nature and purpose of the study was given to both the adolescents and their teachers. Written consent to participate was requested from parents and adolescents. Adolescents with a personal history of cardiovascular disease, who were taking medication at the time of the study, or who were pregnant were excluded. The study protocol was performed in accord with the ethical standards established in the 1961 Declaration of Helsinki (as revised in Hong Kong in 1989 and in Edinburgh, Scotland, in 2000) and was approved by the Review Committee for Research Involving Human Subjects of the Hospital Universitario Marques de Valdecilla, Santander.

Before any testing was performed, the parents completed a questionnaire, part of which addressed the adolescent’s previous and current health status. Socioeconomic status was also assessed by questionnaire completed by the adolescents in whom both homocysteine levels and MTHFR genotypes were measured. Adolescents were in- terested to understand the relative influence of modifiable factors on homocysteine levels from an early age.

PHYSICAL EXAMINATION

Anthropometric measurements were obtained as described elsewhere. In brief, skinfold thickness was measured to the nearest 0.2 mm at the biceps, triceps, subscapular, suprailiac, thigh, and calf on the left side of the body. The sum of the 6 skinfold thicknesses was used as an indicator of body fat. These measurements correlate highly with measured body fat percentage in adolescents of similar ages as measured with dual-energy x-ray absorptiometry.

Identification of pubertal stage was assessed according to the method of Tanner and Whitehouse. Self-reported breast development in adolescent girls and genital development in adolescent boys was used for pubertal stage classification.

MEASUREMENT OF CVF

Cardiovascular fitness was assessed by the 20-m shuttle run test as previously described. In brief, participants were required to run between 2 lines 20 m apart while keeping pace. Running pace was determined by audio signals emitted from a prerecorded cassette tape. The initial speed was 8.5 km/h, which was increased by 0.5 km/h per minute (1 minute equal to 1 stage). The tape used was calibrated over 1 minute. Subjects were instructed to run in a straight line, to pivot on completing a shuttle, and to pace themselves in accord with the audio signals. The test was finished when the subject failed to reach the end lines concurrent with the audio signals on 2 consecutive occasions or when the subject stopped because of fatigue. All measurements were carried out under standardized conditions on an indoor rubber-floor gymnasium. Constant vocal encouragement was given to participants throughout the test. All participants were familiar with the test because the 20-m shuttle run test is one of the fitness tests included in the physical education curriculum in Spain. Adolescents were instructed to abstain from strenuous exercise in the 48 hours preceding the test.

Cardiovascular fitness was considered as the number of stages completed (precision of 0.5 steps) for being the most direct measurement obtained. Moreover, for the purpose of comparing the results with those of previous publications, maximal oxygen consumption (VO_max, milliliters per kilogram per minute) was estimated by the Leger equation:

\[ VO_{\text{max}} = \frac{31.025 + \left[ \frac{3.385 - 3.248A}{1000} \right] + 1.536SA}{80^\circ} \times \text{number of stages completed} \]

The reliability and validity of this test has been shown in young persons.

HOMOCYSTEINE, SERUM FOLIC ACID, AND VITAMIN B12 ASSAYS

With the subject in the supine position, blood samples were obtained by venipuncture after an overnight fast, using vacuum tubes (Vacutainer; Becton, Dickinson and Co, Franklin Lakes, NJ), and placed on ice immediately. The fasting state was verbally confirmed by the subject before blood sampling. All samples were processed within 1 hour by centrifugation, divided into aliquots, and the portions stored at –80°C until withdrawn for analysis.

Homocysteine in acidified citrated plasma was assayed using a fluorescence polarization immunoassay on an IMx unit (Abbott Laboratories, Abbott Park, Ill). Serum folic acid and vitamin B12 levels were measured using the fluorometric method with an IMx automatic analyzer (Abbott Laboratories).

MTHFR GENOTYPING

Total blood DNA was extracted and purified from 500 µL of whole blood anticoagulated with EDTA using the Quiagen procedure described by Higuchi. Genotyping of the 677C>T variant in the human MTHFR gene was performed by means of polymerase chain reaction and allele-specific restriction digestion of the amplified products with the restriction enzyme HinfI (GE Healthcare, Buckinghamshire, England), as previously described by Froost et al.

STATISTICAL ANALYSIS

Data are given as mean ± SD unless otherwise indicated. After serum folic acid and vitamin B12 concentrations were normalized by natural logarithm transformation, all of the residuals showed a satisfactory pattern.

The effect on homocysteine levels of sex and MTHFR 677C>T were analyzed by 1-way analysis of variance because there was a significant interaction between sex and MTHFR
**Fully-formed and pubic hair is adult in quantity and type, forming the classical upside-down triangle common in women.**

Age is 13 to 14 years. The first menstrual period usually occurs sometime during stage 4 or 5, usually at around 12 or 13 years. Tanner stage 5, a girl’s breasts are noticeable growth of pubic hair, now in the triangular shape of adulthood; underarm hair growth is noticeable; breasts begin to take on a “mound” form; average height increases by about 3 inches (7.6 cm) and weight increases by about 70 pounds (32 kg) from Tanner stages 1 to 5.

Tanner stage 4: breast buds become larger and pubic hair growth continues but is mostly in the center and does not extend out to the thighs or upward. Tanner stage 3: breast buds become larger and pubic hair growth continues but is mostly in the center and does not extend out to the thighs or upward.

**RESULTS**

Both homocysteine levels and the MTHFR genotype were measured in 156 adolescents (76 boys and 80 girls). Of these, 23% of the adolescents refused to continue the 20-m shuttle run test because of discomfort or distress, and their results are not included in the final data sample. The observed power for the sample size was 0.40. Pubertal stage was obtained from 96% of the subjects, and skinfold thickness data from 94%. Birth weight, socioeconomic status, and smoking status were available for 93%, 87%, and 71% of the subjects, respectively.

The descriptive characteristics of the study sample are given in Table 1. Adolescent boys were significantly heavier and taller than adolescent girls, and girls had significantly higher skinfold thicknesses. Adolescent boys had significantly higher skinfold thicknesses. Adolescent girls had significantly higher skinfold thicknesses. Adolescent girls had significantly higher skinfold thicknesses.
higher levels of homocysteine, lower levels of serum vitamin B₁₂, and significantly higher CVF levels (Table 1).

Mean values for homocysteine levels were significantly higher in the CT and TT genotype subgroups compared with the CC genotype subgroup in adolescent boys (CC, 61.6±10.0 mg/L [8.3±1.4 µmol/L]; CT, 81.9±40.0 mg/L [11.1±5.4 µmol/L]; TT, 94.5±40.5 mg/L [12.8±5.5 µmol/L]; CT vs CC, \( P = .01 \); TT vs CC, \( P = .003 \)) whereas in adolescent girls, mean values for homocysteine were significantly higher in the TT subgroup compared with the CC and CT subgroups (CC, 55.5±16.8 mg/L [7.5±2.3 µmol/L]; CT, 61.6±12.7 mg/L [8.3±1.7 µmol/L]; TT, 75.3±16.6 mg/L [10.2±2.2 µmol/L]; \( P = .001 \)). Bivariate correlations between homocysteine levels and the studied independent variables are given in Table 2.

### RELATIONS BETWEEN HOMOCYSTEINE LEVELS AND CVF CONTROLLING FOR DIFFERENT CONFOUNDERS AND SEPARATED BY GENDER

The results of the regression models using the homocysteine level as the outcome variable are given in Table 3. Variation in homocysteine levels was significantly explained by CVF (expressed as number of stages completed) in female adolescents after controlling for age, pubertal stage, birth weight, smoking status, socioeconomic status, and the sum of 6 skinfold thicknesses (model 1). Additional adjustments for serum folic acid and vitamin B₁₂ levels (model 2), and MTHFR 677C>T genotype (model 3) further strengthened the association between the homocysteine level and CVF in adolescent girls. No significant association was found between the homocysteine level and CVF in adolescent boys. The results did not change when the analyses were performed with CVF expressed as VO₂max, or speed (data not shown).

### COMMENT

The results of this study suggest that CVF is negatively associated with homocysteine levels in female adolescents but is not associated with homocysteine levels in male adolescents. The results also suggest that homocysteine levels are higher in adolescent boys than in adolescent girls, that serum folic acid and vitamin B₁₂ levels are negatively associated with homocysteine levels, and that MTHFR 677C>T genotype has an important role in homocysteine levels. To our knowledge, there are no other available data on the association of homocysteine levels with CVF in adolescents.

Cardiovascular fitness is a direct marker of physiologic status and reflects the overall capacity of the cardiovascular and respiratory systems and the ability to carry out prolonged strenuous exercise.⁵⁹ In theory, disturbances in the peripheral tissues and related vasculature or in the coronary arteries and the heart may decrease CVF. High CVF levels during childhood and adolescence have been associated with a healthier metabolic profile during these years.²⁰,²⁶ Moreover, CVF has recently been associated with arterial compliance in children aged 9 to 11 years, which supports the concept that CVF may exert a protective effect on the cardiovascular system from an early age.⁴⁶ It is biologically plausible that a high CVF level provides more health protection than a low CVF level, even in healthy adolescents, as has been found in adults.²⁴,²⁵

Homocysteine is metabolized to homocysteine thiolactone by methionyl transfer RNA synthetase. Homocysteine thiolactone acylates lysine residues of proteins, a process called protein homocysteinylation.⁴⁷ Protein homocysteinylation is a possible mechanism of homocysteine-related protein damage, which in conjunction with the increased oxidative stress and endothelial damage seen in subjects with elevated homocysteine levels may result in impaired CVF.⁹ However, this cannot explain why the association between the homocysteine level and CVF is

### Table 2. Bivariate Correlations Between Homocysteine Level* and Independent Variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Adolescent Boys</th>
<th>Adolescent Girls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.12</td>
<td>0.08</td>
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<tr>
<td>Tanner stage</td>
<td>−0.13</td>
<td>−0.04</td>
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<tr>
<td>Birth weight</td>
<td>−0.26</td>
<td>−0.20</td>
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<tr>
<td>Body fat</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>Serum folic acid level</td>
<td>−0.82†</td>
<td>−0.70†</td>
</tr>
<tr>
<td>Serum vitamin B₁₂ level†</td>
<td>−0.18</td>
<td>−0.02</td>
</tr>
<tr>
<td>Cardiovascular fitness</td>
<td>0.05</td>
<td>0.03</td>
</tr>
<tr>
<td>Socioeconomic status</td>
<td>0.03</td>
<td>0.14</td>
</tr>
</tbody>
</table>

*Natural log-transformed values were used in the analysis
†\( P < .001 \)
‡\( P = .02 \)
§\( P < .02 \)

### Table 3. Association of Cardiovascular Fitness (Expressed as Number of Stages Completed) With Homocysteine Level*

<table>
<thead>
<tr>
<th>Model†‡</th>
<th>( \beta )</th>
<th>95% CI</th>
<th>( P ) Value</th>
<th>( R^2 ) Value</th>
<th>( sr )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adolescent boys</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>0.13</td>
<td>−0.06 to 0.09</td>
<td>.64</td>
<td>.10</td>
<td>0.09</td>
</tr>
<tr>
<td>Model 2</td>
<td>0.14</td>
<td>−0.04 to 0.07</td>
<td>.50</td>
<td>.41</td>
<td>0.09</td>
</tr>
<tr>
<td>Model 3</td>
<td>0.12</td>
<td>−0.03 to 0.06</td>
<td>.51</td>
<td>.51</td>
<td>0.08</td>
</tr>
<tr>
<td>Adolescent girls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>−0.40</td>
<td>−0.13 to 0.001</td>
<td>.05</td>
<td>.16</td>
<td>−0.36</td>
</tr>
<tr>
<td>Model 2</td>
<td>−0.40</td>
<td>−0.12 to 0.02</td>
<td>.006</td>
<td>.64</td>
<td>−0.36</td>
</tr>
<tr>
<td>Model 3</td>
<td>−0.40</td>
<td>−0.11 to 0.02</td>
<td>.007</td>
<td>.65</td>
<td>−0.35</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; sr, semipartial correlation.
*Homocysteine and serum vitamin B₁₂ values were natural log-transformed.
†Controlled confounders: Model 1: age, pubertal stage, birth weight, smoking status, socioeconomic status, and sum of 6 skinfold thicknesses. Model 2: model 1 plus serum folic acid and serum vitamin B₁₂ values. Model 3: model 2 plus MTHFR 677C>T genotype.
found only in female adolescents. Our findings support those of a previous study that examined the relationship between homocysteine levels and CVF in adults. Kuo et al showed that high homocysteine levels were negatively associated with estimated CVF in women. However, they did not find any association in men, which is in accord with our results. These results suggest that sex hormones may have a role in mediating the CVF–homocysteine association, exerting different effects in female and male subjects; however, further studies to determine whether this is the case are needed. One longitudinal study observed 499 independent community-dwelling elderly persons for 3 years and found that those with elevated homocysteine levels were at increased risk of decline in physical function. However, CVF data were not provided and a comparison by sex was not performed.

None of the previous studies included the MTHFR 677C>T genotype, which affects homocysteine levels. Balasa et al found that the MTHFR 677C>T polymorphism was an independent determinant of homocysteine levels in 197 healthy US children aged 6 months to 16 years. Similarly, Papoutsakis et al reported in a sample of healthy Greek children that the TT genotype was associated with homocysteine concentrations. Homocysteine levels in our study sample were significantly higher in the MTHFR 677C>T and TT genotype subgroups compared with the CC subgroup in adolescent boys, whereas in adolescent girls, mean values of homocysteine were significantly higher in the TT genotype subgroup compared with the CC and CT genotype subgroups.

In the present study, CVF was objectively measured by the 20-m shuttle run test. We did not have a direct measurement of VO₂max, the most valid method of measuring CVF. However, from a practical point of view, field tests may be a better option than laboratory testing, especially in epidemiologic studies, because a large number of subjects can be tested at the same time, which enhances the motivation of the participants, and the tests are simple, safe, and often the only feasible choice, especially in school settings. The 20-m shuttle run test meets these criteria. Cardiovascular fitness was considered as the number of stages completed in the 20-m shuttle run test. However, CVF estimated from the Leger equation (VO₂max, milliliters per kilogram per minute) was also provided for the purpose of making comparisons with other studies possible. When the analyses were performed using VO₂max or speed (kilometers per hour) rather than the number of stages as the measurement of CVF, similar results were obtained.

Results from cross-sectional studies have shown associations between homocysteine levels and lifestyle-related factors. However, findings are different when analyzed prospectively. Duncan et al found that 6 months of exercise increased homocysteine levels in sedentary adults, whereas Randeva et al showed that 6 months of sustained brisk walking for 20 to 60 minutes 3 days a week significantly decreased homocysteine levels and increased CVF in young overweight and obese women with polycystic ovary syndrome, a group at increased risk of premature atherosclerosis. Similarly, a weight-reduction program that included physical activity had a positive effect on the homocysteine levels in obese children. Together, these results suggest that modifications in lifestyle-related factors may influence homocysteine levels in a different manner in children and adolescents than in adults.

The results from the present study should be interpreted with caution because of the limitations of the cross-sectional design; that is, direction of causality cannot be determined. Elevated homocysteine levels may be simply a marker of an unhealthy lifestyle that is associated with poor exercise capacity. The relationship between homocysteine levels and CVF should be studied prospectively. It must be borne in mind that the subjects in this study were healthy adolescents with no previously diagnosed cardiovascular disorders. Also, our study included a moderate number of participants. The observed power for the sample size was low (0.40), which may have masked the association between CVF and homocysteine levels in the adolescent boys. This warrants further investigation. However, we believe that covariates that may confound the measures of association in our study were appropriately considered and controlled for.

The results of this study suggest that CVF is negatively associated with homocysteine levels in adolescent girls after controlling for potential confounders including the MTHFR 677C>T genotype. These results should stimulate a debate on whether the metabolism of homocysteine could be one way in which the benefits of high CVF levels are exerted.

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REFERENCES


Why is the Game Called Cat’s Cradle?

The term was originally cratch-cradle, and cratch is from Middle English creche, meaning a rack in which hay is put for cattle. The first figure created with the string in cat’s cradle looks like a cratch.

—From Why Do We Say It? Castle Books, 1985

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