Cardiorespiratory fitness modifies the association between the UCP3-55C>T (rs1800849) polymorphism and plasma homocysteine in Swedish youth

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1. Introduction

Epidemiologic and clinical evidence show that hyperhomocysteinaemia is an independent, modifiable risk factor for atherosclerosis and cardiovascular disease [1]. The mechanisms whereby homocysteine causes endothelial injury and vascular disease are not fully understood, though a link between homocysteine and its intermediates and altered DNA methylation pattern was recently postulated, together with the involvement of epigenetic mechanisms causing inhibition of transmethylation reactions [1]. Plasma homocysteine levels are influenced by genetic and non-genetic factors. Homozygosity for the common 677C>T variant in the methylenetetrahydrofolate reductase (MTHFR) gene [2] as well as cigarette smoking, excessive alcohol intake, lack of physical activity and nutritional deficiencies in folate and vitamin B12 are associated with elevated homocysteine [3–5]. A significant negative association was also reported between cardiorespiratory fitness and plasma homocysteine in adult women [6] and female adolescents [7], though this finding was not corroborated in adult men [6] or in children [8].

Uncoupling protein 3 (UCP3) is a mitochondrial anion carrier protein that is highly expressed in skeletal muscle [9]. It diminishes mitochondrial superoxide production and may protect against oxidative endothelial damage [9,10]. In middle-aged men, homozygosity for the T allele of the -55C>T polymorphism in the promoter region of UCP3 gene, which is likely associated with reduced gene expression, accelerates the onset of diabetes [11]. This genetic variant is also associated with adult abdominal fat distribution [12] and obesity [13,14]. Other genetic variants of the UCP3 gene, i.e., rs591758, rs647126, and
rs1800006, have also been associated with type 2 diabetes risk [15].

Whether polymorphisms in the UCP3 gene are associated with plasma homocysteine levels in youth is not known and the putative modifying role of cardiorespiratory fitness on this association has not been elucidated. This question is of clinical interest owing to the fact that cardiorespiratory fitness is a potent cardiovascular risk factor in all age-groups [16] and high cardiovascular fitness during childhood years is associated with healthier cardiovascular profile later in life [17].

The aims of this study were (i) to analyse the association between polymorphisms in the UCP3 gene and plasma homocysteine levels in a cohort of Swedish children and adolescents and (ii) to examine whether cardiorespiratory fitness modifies this association.

2. Materials and methods

Children and adolescents were participants in the Swedish part of the European Youth Heart Study (EYHS), a school-based, cross-sectional study of risk factors for cardiovascular disease [18]. The present study population comprised 267 Swedish children (age range 8–10 years) and 305 adolescents (14–16 years). The study was approved by the Research Ethics Committees of Örebro County Council and Huddinge University Hospital. We obtained written informed consent from the parents of the children and adolescents, and from the adolescents themselves.

We measured height and weight by standardized procedures. Total body fat was expressed as the sum of five skinfold thicknesses, i.e. caliper at the biceps, triceps, subscapular, suprailiac and triceps surae, which we measured using the methods described by Lohman et al. [19]. We assessed pubertal development according to Tanner and Whitehouse.

Socioeconomic status was assessed via questionnaire and defined by the maternal educational status, coded as 0 (below university education) and 1 (university education). We assessed levels of physical activity with an activity monitor (MTI model WAM 7164, Manufacturing Technology Inc., Shalimar, Florida, formerly known as Computer Science and Applications Inc.) attached on the right hip of participants as detailed elsewhere [20]. We expressed physical activity as total counts recorded divided by total daily registered time (counts/min).

Homocysteine was assayed in acidified citrated plasma using a fluorescence polarization immunoassay on an IMX™ unit (Abbott Laboratories, Abbott Park, IL, USA) [8]. Folate and vitamin B12 intake were assessed by an interviewer mediated 24-h recall as described elsewhere [8].

The UCP3 polymorphisms rs1800849 (also known as UCP3-55C>T), rs1800006, rs2075577, rs647126, and rs591758 (enumerated in their respective order 5′→3′ on chromosome 11) were genotyped by DNA sequencing using pyrosequencing assays (Table 1). Genotyping of the 677C>T polymorphism in the MTHFR gene (rs1801133) was also performed on the pyrosequencing platform, using a standard protocol (Biotech AB, Uppsala, Sweden [www.biotech.com]) [21]. Haplotypes of the UCP3 two-locus systems consisting of UCP3-55C>T plus each one of the other four loci were constructed manually and the assignments, including the few ambiguous diplotypes, were confirmed using PHASEver2.1 [22,23]. Long-range haplotypes based on all 5 studied loci, and the corresponding diplotypes, were also constructed using PHASEver2.1.

Cardiorespiratory fitness was assessed with a maximum cycle-ergometer test on an electronically braked cycle ergometer (Monark 829E Ergomedic, Vansbro, Sweden) and was expressed as maximum oxygen uptake (VO2max) relative to body mass (mLO2/kg/min) [24].

2.1. Statistical analyses

All analyses were performed using the Statistical Package for Social Sciences (SPSS, v. 16.0 for WINDOWS; SPSS Inc., Chicago). Differences in homocysteine levels between genotypes of all five studied UCP3 polymorphisms were analysed (as dominant traits) by one-way analysis of covariance (ANCOVA) after adjusting for gender, age, pubertal status (model 1), folate and vitamin B12 intake (model 2) and MTHFR 677C>T polymorphism (as recessive trait, model 3). The effects of diplotypes of the two-locus haplotypes and of the five-locus “long-range” haplotype were tested in the same way.

We assessed the putative modifying role of cardiorespiratory fitness by inserting the interaction term into the models. Cardiorespiratory fitness was entered into the model as discrete variable (low, moderate and high, which refers to 1st, 2nd to 3rd, and 4th, respectively). Low, moderate and high cardiorespiratory fitness levels in girls were <39.2, 39.2–51.2 and >51.2 mLO2/kg/min, respectively. The corresponding values for female adolescents were <39.6, 39.6–51.3 and >51.3 mLO2/kg/min, respectively. Low, moderate and high cardiorespiratory fitness in boys were <39.6, 39.6–51.4 and >51.4 mLO2/kg/min, respectively. The corresponding values for male adolescents were <39.7, 39.7–54.6 and >54.6 mLO2/kg/min, respectively. We adjusted multiple comparisons for mass significance [25].

<table>
<thead>
<tr>
<th>SNP</th>
<th>PCR primer sequence 5′→3′</th>
<th>Annealing temp (°C)</th>
<th>MgCl2 conc. (mmol/L)</th>
<th>Size (bp)</th>
<th>Sequencing primer 5′→3′</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCP3-55C&gt;T</td>
<td>rs18000849</td>
<td>56</td>
<td>1.5</td>
<td>116</td>
<td>CTGGCACTTGCTTTATA</td>
</tr>
<tr>
<td>UCP3</td>
<td>rs1800006 (T&gt;C)</td>
<td>60</td>
<td>1.5</td>
<td>119</td>
<td>TGTACACTGCTTGATGGA</td>
</tr>
<tr>
<td>UCP3</td>
<td>rs2075577 (T&gt;C)</td>
<td>60</td>
<td>1.5</td>
<td>207</td>
<td>GAGAAAGCTGCTGAC</td>
</tr>
<tr>
<td>UCP3</td>
<td>rs647126 (A&gt;G)</td>
<td>60</td>
<td>1.5</td>
<td>222</td>
<td>TCTGTTGCTGGGT</td>
</tr>
<tr>
<td>UCP3-UCP2</td>
<td>rs591758 (C&gt;G)</td>
<td>60</td>
<td>1.5</td>
<td>109</td>
<td>AGATGACCAGCCTGA</td>
</tr>
</tbody>
</table>

PCR primers and sequencing primers were designed using the Assay Design Software version 1.0.6 Biotech AB, Uppsala Sweden. The reactions for rs1800849, rs1800006 and rs577158 are reversed assays (interrogating the noncoding strand); PCR amplification for the pyrosequencing assay was performed with the TopTag Polymerase Kit (Qiagen Inc., Valencia, CA, USA). The reaction mixture (50 μL total volume) contained 0.2 μmol/L each of the sense and antisense primer, 1.25 units of TopTag polymerase, 2.0 mmol/L MgCl2, and 0.2 mmol/L each of dCTP, dATP, dTTP and dGTP; about 20 ng of the purified patient genomic DNA was added as template. After PCR, the samples were prepared according to a standard protocol using the Vacuum Prep Workstation (Biotech AB, Uppsala, Sweden): Sequencing was performed using a Pyro Gold Reagent Kit and a PSQ™ 96MA system (Biotech AB). The PSQ™ 96MA 2.0.2 software was used to optimise the nucleotide addition orders (available upon request). Results were automatically analysed using the PSQ™ 96MA 2.0.2 software.

\[\text{VO}_2\text{max} / \text{mLO}_2 / \text{kg/min} \]

\[\text{Urea} / \text{mmol/L} \]

\[\text{Triglyceride} / \text{mmol/L} \]

\[\text{HDL-C} / \text{mmol/L} \]

\[\text{LDL-C} / \text{mmol/L} \]

\[\text{Blood pressure} / \text{mmHg} \]

\[\text{Heart rate} / \text{bpm} \]

\[\text{Physical activity} / \text{counts/min} \]

\[\text{Homocysteine} / \mu\text{mol/L} \]

\[\text{Folate} / \mu\text{g/L} \]

\[\text{Vitamin B12} / \text{nmol/L} \]

\[\text{Cardiorespiratory fitness} / \text{mLO}_2 / \text{kg/min} \]

\[\text{Pubertal status} \]

\[\text{Gender} \]
Table 2
Table 3
3. Results
All five studied UCP3 polymorphic loci, and the MTHFR 677C>T locus, were in agreement with the Hardy–Weinberg equilibrium (all \( \chi^2 < 3.8 \)). The frequencies of the minor alleles (q) of the UCP3 polymorphisms in this Swedish population sample were 0.280, 0.269, 0.438, 0.470, and 0.377 for rs1800849, rs1800006, rs2075577, rs647126, and rs591758, respectively.

After adjusting for age, gender, pubertal status, folate and vitamin B12 intake and MTHFR 677C>T polymorphism, we observed that participants who were homozygous or heterozygous for the T allele of the UCP3-55C>T polymorphism had significantly higher homocysteine levels than those carrying the wildtype (CC) genotype (Table 2; \( P = 0.042 \)); thus, we studied the associations between -55C>T (rs1800849) polymorphism and homocysteine levels by levels of cardiorespiratory fitness (7.88 \( \pm 0.18 \) \( \mu \)mol/L vs. 8.36 \( \pm 0.19 \) \( \mu \)mol/L for CC vs. CT+TT, respectively, \( P = 0.075 \)). The results persisted after further adjustment for physical activity.

The effect of the UCP3-55C>T polymorphism on homocysteine levels was attenuated after further adjusting for cardiorespiratory fitness (7.88 \( \pm 0.18 \) \( \mu \)mol/L vs. 8.36 \( \pm 0.19 \) \( \mu \)mol/L for CC vs. CT+TT, respectively, \( P = 0.075 \)). The results persisted after further adjustment for physical activity. There was a significant interaction effect of cardiorespiratory fitness \( \times \) UCP3-55C>T polymorphism (\( P = 0.042 \)); thus, we studied the associations between UCP3-55C>T and homocysteine levels by levels of cardiorespiratory fitness (low, moderate and high) after adjusting for gender, pubertal status, folate and vitamin B12 intake and MTHFR 677C>T polymorphism. We observed that the effect of UCP3-55C>T polymorphism on homocysteine levels persisted in youth with low
cardiorespiratory fitness (Fig. 1), whereas it was abolished in those with moderate or high cardiorespiratory fitness. Further adjustment for total body fat (sum of five skinfolds), socioeconomic status, or physical activity did not materially affect the results. Similarly, the results persisted when adjusting for age instead of pubertal status. The analyses were repeated using different common ways of defining cardiorespiratory fitness, i.e. thirds, fifths, and the influence of UCP3-55C>T polymorphism on homocysteine was in all cases greater in low fit youth.

4. Discussion

The results of the present study indicate that the UCP3-55C>T polymorphism (rs1800849) affects homocysteine levels in Swedish children and adolescents, with the levels being higher in those individuals who are homozygous or heterozygous for the T (minor) allele. In contrast, we did not find an effect of the other UCP3 variants studied. The haplotype data confirmed that the 5’-end of the UCP3 gene is a genetic predictor affecting homocysteine levels. The effect may reside in the UCP3-55C>T locus itself or possibly in another locus in its vicinity. We also observed that cardiorespiratory fitness modifies the association between the UCP3-55C>T polymorphism and homocysteine so that the negative effect of the T allele does not persist in individuals with moderate to high cardiorespiratory fitness. The homocysteine-raising effect of the T allele may be too small to warrant introducing UCP3-55C>T genotyping in the clinical diagnostic workup of patients with hyperhomocysteinemia. However, it should be included in all future epidemiological studies on homocysteine levels since we have shown that it is characterised by significant gene–environment interaction upon homocysteine levels which needs to be addressed in further studies.

Elevated levels of homocysteine in plasma promotes the formation of reactive oxygen species (ROS) [26,27]. The higher homocysteine levels observed in participants with the CT and TT genotype for the UCP3-55C>T polymorphism could be related to a lower UCP3 expression, and thus lower UCP3 antioxidant activity, associated with the T allele. On the other hand, exercise, when practiced in a moderate basis, is known to increase the expression of antioxidant enzymes, conferring greater protection against ROS [28].

The UCP3-55C>T polymorphism (rs1800849) also affects adenosine triphosphate (ATP) production in the mitochondria [29]. It was hypothesized that the UCP3-55 T allele may lead to increased levels of homocysteine by a mechanism linked to a reduced ATP synthesis in the mitochondria, through its effect on the transmembrane proton gradient [29,30]. Reduced mitochondrial ATP production could in addition be a cause of reduced folate levels regionally in the body and thereby to higher homocysteine levels, as suggested by a genetic disease recently described in a child with a hereditary encephalopathy [31]. In clinical studies, the T allele has so far not been studied with respect to homocysteine levels, but there are previous studies showing that it is associated to the development of diabetes [11] and obesity [13,14].

Our results highlight the importance of having at least moderate levels of cardiorespiratory fitness to mitigate the association between the UCP3-55C>T polymorphism and plasma homocysteine during the first decades of life. Exercise interventions are useful to improve cardiorespiratory fitness and reduce visceral fat, insulin resistance, and triglyceride levels among youth [32,33]. Thus, it is biologically plausible that moderate to high levels of fitness confers a protection against the effect of the UCP-55C>T polymorphism on homocysteine levels. There is compelling evidence from epidemiological studies that moderate-high levels of cardiorespiratory fitness during childhood and adolescence are associated with a healthier cardiovascular profile during these years and later in life [16,17].

Interestingly, the fitness categories used in the present study are similar to those recently suggested elsewhere [34], which have been associated with an improved cardiovascular profile. This supports the existence of a cardiorespiratory fitness value linked to a more favourable cardiovascular profile in youth, as it has been shown in adults. From a public health point of view, these findings emphasize the importance of avoiding low fit states in youth for purposes of early cardiovascular disease prevention. Clinical screening and monitoring as well as epidemiologic surveillance of youth who fail to meet the health-related fitness standards can help identifying youth at increased risk of cardio-metabolic diseases who could benefit from intervention programs.
Conflict of interests

None.

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References


