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Relation of Total Homocysteine and Lipid Levels in Children to Premature Cardiovascular Death in Male Relatives

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Abstract

We assessed the relative importance of lipid, apo B, lipoprotein(a)[Lp(a)], and total homocysteine (tHcy) levels in children in relation to premature cardiovascular disease in family members. Parents of 381 girls and 375 boys age 8-12 y completed family history questionnaires. Nonfasting serum lipids and lipoproteins and plasma tHcy and cysteine levels were measured in the children. Serum folate and vitamin B_{12} levels were determined in a random subsample of 23% of the children, who participated in a food frequency interview. Children whose parents reported hypercholesterolemia had higher total and non-HDL cholesterol and apo B levels than the rest, but these levels were not associated with cardiovascular disease. tHcy levels were similar in girls and boys. tHcy was higher in children whose father, grandfather, or uncle died at age ≤55 y of myocardial infarction or sudden cardiac arrest(n = 42) than in control children [5.92 μmol/L (95% confidence interval [CI] of 5.47-6.36) *versus* 5.25 μmol/L (95% CI, 5.16-5.34)], also after adjustment for socioeconomic group. Intake and serum levels of vitamin B_{12} and folate were within recommended or reference ranges. In a stepwise multiple regression analysis, serum folate (negative correlation), plasma creatinine, and sugar intake as percent of dietary energy(positive correlations) were significantly associated with tHcy (multiple$r$ = 0.44, adjusted r^2 = 18%; 95% CI, 5-30%). Our data show that a modest elevation in tHcy in children was related to premature cardiovascular death in their male relatives and may partly account for the contribution of family history to risk of cardiovascular disease. tHcy

may be modifiable through the diet, even in children with apparently adequate vitamin nutriture.

Abbreviations: tHcy, total homocysteine; **tCys,** total cysteine; **Lp(a),** lipoprotein(a); **CI,** confidence interval

Children whose relatives have experienced premature cardiovascular disease may have higher total or LDL cholesterol[1-4], lower HDL cholesterol[3, 5], higher apo B [4, 6, 7], or Lp(a) [8-11], lower apo A-I[5-8], or lower LDL cholesterol/apo B ratios [12] than controls. Several expert groups recommend that the family history should guide screening for hypercholesterolemia during childhood[13-15]. However, other reports have shown that targeted screening is insensitive and nonspecific[3, 4, 16-19], leading some authors to advocate no screening, universal screening, or screening only in families with known familial hypercholesterolemia[20-22].

In a cross-sectional survey in 15 countries, Norwegian children had the second highest cholesterol levels after Finland, but Norway ranked only fourth in coronary artery disease mortality [23], indicating that other factors modify the relationship between childhood lipids and cardiovascular disease. Increased plasma tHcy is an independent risk factor for premature cardiovascular disease, even in subjects whose tHcy levels are within the reference range [24-26]. tHcy is influenced by age, sex, vitamin B_{12} and folate status, genetic factors, renal function, and possibly the synthesis of creatinine[24, 27-29]. Plasma tCys (the concentration of cysteine which can be measured after chemical reduction of disulfide bonds), another sulfur-containing amino acid, may also be associated with vascular disease [30].

The aim of this study was to assess the importance of lipid, apo B, Lp(a), tHcy (the concentration of homocysteine which can be measured after chemical reduction of disulfide bonds), and tCys levels in children, in relation to familial premature cardiovascular disease, and to examine dietary determinants of tHcy in children.

METHODS

Primary schools in Oslo with ≤15% of students of non-Norwegian origin were randomly sampled from two groups: one whose population's mean income was similar to the mean of the city, and the other with an income 25% above the mean. Grades 2-5 (ages 8-12 y) from four schools in the high income and six schools in the mean income group participated. Consent was required of a parent and the child. The regional ethics committee allowed the results of the blood test to be communicated to the parents only in the case of familial hypercholesterolemia. Of all students, 40% agreed to participate (33% from the high income group and 48% from the middle income group).

Family history. The questionnaire followed the format previously described [23], requesting information from each natural parent on personal and family history of hypercholesterolemia and cardiovascular disease, the age of onset and, if relevant, age at death. Reports of death at or below age 55 y in male relatives (the child's father, uncle, or grandfather) that were attributed to a cardiovascular cause were checked by contacting the parent to verify the age at death, cause of death, and that the diagnosis was provided by a physician. Hospital, autopsy, or

physician records were examined when available (27 of 51). Sudden cardiac death attributed to an atherosclerotic process and deaths as a result of myocardial infarction were included in the premature death group.

Of the 51 initial reports, one death was a suicide, one occurred at age 56 y, one was due to cardiomyopathy, one was due to an abdominal aneurysm, one was due to cancer, one was of unknown etiology, and three could not be traced, leaving 42. The group included three fathers and four uncles; the rest were grandfathers.

Blood sampling and biochemical analyses. The children were asked to eat a low calorie breakfast on the day of testing. A dietary recall from children (n = 108) at a randomly chosen school showed that the mean intake at breakfast was 213 kcal, including 31% from fat, 15% from protein, and 54% from carbohydrate.

Blood samples were obtained between 0900 h and 12 noon. Serum lipid analyses were performed on the same day, and any leftover serum was stored at-38 °C. EDTA samples were immediately placed on ice. The plasma was separated within 30 min and stored at -20 °C. Analysis of plasma tHcy was completed within 10 mo on all but four samples (insufficient plasma). Serum folate and vitamin B_{12} and plasma creatinine were measured within 10 mo on samples from the children who participated in the dietary interview, when enough serum was available (n = 135).

Serum cholesterol and triglycerides were determined enzymatically (reagents supplied by Boehringer Mannheim AG) on a Hitachi 911 autoanalyzer. HDL cholesterol was measured enzymatically (reagents suppled by Boehringer Mannheim AG) on a Monarch 2000 analyzer after precipitation of apo B-containing lipoproteins with a standard heparin-manganese solution. Apo B was measured by immunoturbidimetric methods (Orion Diagnostica, Finland) on a Monarch 2000 analyzer. Lp(a) was measured by a two-site immunoradiometric assay (Pharmacia Diagnostics, Sweden). Non-HDL cholesterol was calculated by subtracting HDL cholesterol from total cholesterol.

Vitamins B_{12} and folate were measured by radioassay using a commercial kit (Dualcount Charcoal Boil Assay, Diagnostic Products Corporation, Los Angeles, CA). Plasma creatinine was determined according to the method of Jaffe (Technicon, Tarrytown, NY).

Plasma tHcy and tCys were determined using a modification of a fully automated assay based on precolumn derivatization followed by reversed-phase HPLC [(31, 32)]. Plasma tHcy and tCys refer to the levels of homocysteine and cysteine available for derivatization with monobromobimane after treatment of native plasma with sodium borohydride. The precision (between-day coefficient of variation) of the assay is approximately 2%.

Dietary assessment. A random sample of children and their mothers from the high income (22%) and from the mean income schools (24%) was interviewed by a dietician about the child's dietary habits during the past year. The interview was based on a quantitative food frequency questionnaire developed at the Institute for Nutrition Research in Oslo[(33)]. Intake of vitamin supplements was added, if vitamins were taken at least three times a week for at least 6 mo a year. At the interview, the educational level of the parents was recorded, and the child's height and weight were measured.

Statistical analysis. Questionnaires were incomplete or missing from 31 fathers and 15 mothers, due to adoption or to lack of contact with the parent. When both questionnaires were missing, or if one questionnaire was missing and the available questionnaire did not indicate the presence of disease, the child was excluded from analyses that included the family history.

Assessment of statistical power showed that over 100 persons in each group were required to detect a 0.5 mmol/L difference in total cholesterol between the risk group and the controls with both α and β = 0.05. The prevalence of premature cardiovascular disease or death in relatives was expected to be about 15%, thus, 700 participants were required.

Statistically significant differences in risk factors between groups were calculated by unpaired *t* tests or analysis of variance. Lp(a) levels were log-transformed before analysis of variance was done. For comparison of tHcy levels in the premature death group and controls, socioeconomic group was a covariate in an analysis of covariance. Univariate regression coefficients were calculated for the relation of tHcy and other factors, followed by multiple regression analyses. Probability values ≤0.05 were considered significant. Statistical analyses were performed using Statview and SPSS software packages.

RESULTS

Parental educational level was higher in the high compared with the mean income schools (data not shown), confirming that the groups differed in socioeconomic status. Socioeconomic level was lower in children belonging to the premature death group compared with the controls (p = 0.05; data not shown). Family histories of 78 children from whom blood samples were not obtained did not differ from the participants' histories.

The mean, median, and 90th percentile levels for tHcy for the entire group were 5.3, 5.1, and 6.9 μmol/L, respectively. Children from the high socioeconomic group tended to have higher cholesterol, HDL cholesterol, and non-HDL cholesterol/apo B and lower non-HDL cholesterol, triglyceride, total/HDL cholesterol, tHcy, and tCys levels (Table 1). tHcy levels were similar in girls and boys. tHcy levels were weakly correlated with HDL cholesterol (r = -0.14; p = 0.0001), triglyceride(r = 0.20; p = 0.0001) and apo B levels (r = 0.14; p = 0.003).

Table 1

Lipid and tHcy levels and the family history. Offspring of mothers with hypercholesterolemia (n = 42) had higher levels of total [5.24 ± 0.96 mmol/L (mean ± SD)] and non-HDL cholesterol(3.83 ± 0.90 mmol/L) than those whose parents did not report hypercholesterolemia (reference group, n = 602: total cholesterol, 4.68 ± 0.75 mmol/L and non-HDL cholesterol, 3.26 ± 0.73 mmol/L;p = 0.0001). Levels of apo B and triglycerides were also higher (apo B, 0.76 ± 0.15 g/L *versus* 0.67 ± 0.15 g/L, p= 0.0002; triglycerides, 1.06 ± 0.61 mmol/L *versus* 0.86± 0.54 mmol/L, p = 0.02). Offspring of fathers with hypercholesterolemia (n = 84) had significantly higher total (4.92± 0.68 mmol/L) and non-HDL cholesterol (3.56 ± 0.72 mmol/L) and higher apo B (0.72 ± 0.18 g/L) than the reference group (p = 0.02, 0.002, and 0.005, respectively).

Risk factor levels did not differ between children (n = 128) whose parent, uncle, aunt, or grandparent had premature (≤55 y) myocardial infarction and those without a report of myocardial infarction (n = 602) (data not shown). The addition of relatives with angina pectoris or heart failure to the group with myocardial infarction did not change the results.

Plasma tHcy levels were significantly higher [5.92 (95% CI, 5.47-6.36μmol/L) *versus* 5.25 (95% CI, 5.16-5.34 μmol/L)];Table 2 and Fig. 1) in children in the premature death group compared with the controls, also after adjustment for socioeconomic group (5.88 μmol/L *versus* 5.28 μmol/L;p = 0.002). Lipid and apo B levels did not differ significantly between the groups (Table 2). Level of log-transformed Lp(a) was marginally lower in the children in the premature death group(p = 0.033). The nontransformed levels were 183 ± 271 mg/L*versus* 230 ± 283 mg/L (not significant).


Table 2

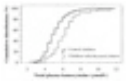
Figure 1

The analysis could not be performed for female relatives because only two reported a grandmother and none a mother or an aunt in the premature death category.

Dietary intake. Dietary variables were similar in the high and middle socioeconomic groups. Mean energy intake was 2204 ± 429 kcal, with 54% ± 4% of calories from carbohydrates (including 11% ± 4% from sugar), 14% ± 2% from protein and 31% ± 4% from fat. Levels of tHcy, but not tCys, were significantly higher in children whose sugar intake was ≥13.4% of energy (highest quartile) than in those whose intake was lower (Table 3), and sugar intake remained associated with tHcy after adjustment for age, sex, and riboflavin, vitamin B_{12} and folate intake, serum folate, plasma creatinine, and body mass index in a multiple regression analysis (standard coefficient = 0.23, p = 0.008).


Table 3

Mean intake of vitamin B_{12} and folate was 7.1 ± 2.3 μg/d and 174 ± 49 μg/d, respectively. In all but one child, intakes of vitamin B_{12} were above the recommended level [34]. Folate intakes for children between 7-10 y were above the recommended level. For children 11-12 y old, individual intakes were lower in 31% than recommendations for age 11-14 y. Intake of folate and vitamin B_{12} was not related to tHcy.

Vitamin and creatinine levels. Serum B_{12} and folate levels were above the lower reference range of the laboratory (120 pmol/L and 6.0 nmol/L, respectively) and were related to the dietary intake of the vitamin (r = 0.18, p = 0.04 and r = 0.24,p = 0.005, respectively). tHcy levels were significantly higher in children in the lowest quartile of serum folate and in the highest quartile of plasma creatinine (Table 3). The correlation between tHcy and serum folate remained significant in a multiple regression analysis including age, sex, and riboflavin, vitamin B_{12}, and folate intake, sugar intake, plasma creatinine, and body mass index (standard coefficient =-0.29; p = 0.0008). In a stepwise multiple regression analysis, serum folate

(negative correlation), plasma creatinine, and sugar intake(positive correlations) were significantly associated with tHcy level(multiple r = 0.44, adjusted r^2 = 18%; 95% CI, 5-30%).

DISCUSSION

Our study shows that plasma tHcy in young children is associated with premature cardiovascular death in male relatives (usually grandfathers). Although total and non-HDL cholesterol, triglyceride, and apo B levels were higher in children whose parents reported hypercholesterolemia, lipid, and lipoprotein levels, including Lp(a), were not associated with premature death.

Elevated tHcy levels have been observed in the adult offspring of men with premature coronary artery disease [(28)], but to our knowledge, no study of tHcy levels in children in relation to familial disease has been published. In about 30% of patients with hyperhomocysteinemia and premature peripheral vascular disease or cerebral occlusive arterial disease, elevated tHcy levels are attributable to so-called thermolabile 5,10-methylenetetrahydrofolate reductase [(35)], caused by a common mutation at the reductase locus [(36)]. Whether a genetic defect underlies the findings in the present study remains to be explored. These findings may explain in part the independent contribution of the family history to the risk of cardiovascular disease[(37)]. Whether hyperhomocysteinaemia in childhood is predictive of future cardiovascular disease cannot be established in the present retrospective study. Future prospective studies should explore the significance of mild hyperhomocysteinemia in childhood.

The mean and 90th percentile levels of tHcy were about 50% of levels in adults [(38)] and were similar to values noted in smaller studies [(39)]. We found that tHcy levels were similar in girls and boys. Levels of tHcy ≥12.0 μmol/L were not observed, indicating that the difference between the groups is not attributable to a few subjects with markedly elevated tHcy levels in the group with familial premature death, as is illustrated in Figure 1. tHcy was 11% higher in the high risk group than in the controls. In the present study, levels of tHcy in the male relatives who died are unknown; thus, a causative relationship between modestly elevated tHcy and risk of cardiovascular disease is not proven. However, our findings are consistent with the concept that modest hyperhomocysteinemia influenced by genetic factors[(35)] is a risk factor for premature cardiovascular disease [(27)]. This concept has been substantiated by two prospective studies, including one from Norway, in which mean levels of tHcy were 6-12% higher in subjects with myocardial infarction than in controls[(25, 40)].

tHcy showed the expected inverse relationship to folate, although not to vitamin B_{12} levels [(24, 29)]. Vitamin B_{12} intake is adequate among most consumers of a nonvegetarian diet, whereas marginal levels of folate are more common [(41, 42)]. Notably, the lowest quartile of serum folate was within the reference range. Similarly, dietary folate appeared to be adequate and was related to serum folate. Although the intake of some of the children age 11-12 y was lower than recommended for ages 11-14 y, intake is intended to increase gradually between ages 10 and 14 y. The association between high sugar intake and elevated tHcy levels may indicate that sugar intake is a surrogate of optimal vitamin nutriture among children, even when vitamin intake appears normal, an observation that has been made in at least one other population[(43)].

Lipids and the family history. In the present study, the family history of cardiovascular disease was not predictive of hypercholesterolemia in the children, confirming findings in several studies in Northern Europe[16, 18, 44]. When screening is limited to children with a positive family history, more than three-fourths of cases may be missed [3, 4, 17-19]. The association between childhood lipids and familial disease varies in different populations, depending on the diet and rate of maturation[45], and may be confounded by female sex[20] and socioeconomic status. High socioeconomic status was associated with higher total cholesterol levels in this study, but is predictive of lower rates of cardiovascular disease [46]. Only upon inspection of apo B and HDL cholesterol levels and total/HDL cholesterol and non-HDL cholesterol/apo B ratios is it apparent that the high socioeconomic group had protective levels or ratios compared with the mean socioeconomic group. However, screening for low HDL cholesterol in prepubertal children is premature, as HDL cholesterol is influenced by life style characteristics and sex hormones [5]. Likewise, the level of Lp(a) appears to increase at least up to age 10 y[10]; thus, our failure to find an association between Lp(a) levels and premature cardiovascular disease is not surprising. Previous studies that showed a relationship between Lp(a) level in offspring and familial cardiovascular disease involved older children[8, 9] or examined different outcomes than the present study [11]. Although Lp(a) levels appeared to be lower in the group with familial premature death, this finding may be due to chance. Apo(a) phenotype, not examined in the present study, appears to provide a more accurate assessment of risk than level of Lp(a)[47].

Although nonfasting levels of risk factors were measured in this study, earlier studies have shown that lipid levels in children are not significantly affected by food intake [48]. Nonfasting triglyceride levels may be more important indicators of cardiovascular risk than fasting levels [49]. tHcy is probably not affected by a small breakfast [50].

We did not verify other cardiovascular events than deaths before age 56. Thus, misclassification of these events may have occurred. The association between hypercholesterolemia and the family history may be stronger if the relative with cardiovascular disease is a parent[2-7], rather than a grandparent[17]. Parental disease was rare in our population, both due to the young age of the children and the selection of mean or high socioeconomic groups. The selection of these groups, the need for venipuncture, and the ethical committee's decision to restrict the parents' access to the results probably contributed to the low rate of participation, but participants did not appear to differ from a group of non-participants.

In conclusion, children who have a male family member who died prematurely of cardiovascular disease may have modestly elevated tHcy levels. These findings may in part explain the independent contribution of the family history to the risk of cardiovascular disease. tHcy level was associated with dietary and socioeconomic characteristics in these children with apparently normal vitamin nutriture.

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