Young adults with family history of coronary heart disease have increased arterial vulnerability to metabolic risk factors. The Cardiovascular Risk in Young Finns Study.

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ONLINE SUPPLEMENT
METHODS

Subjects

The Cardiovascular Risk in Young Finns Study is an on-going 5-centre follow-up study of atherosclerosis precursors of Finnish children and adolescents. The study has been carried out in all five Finnish university cities with medical schools (Helsinki, Kuopio, Oulu, Tampere, Turku) and their rural surroundings. In 1980, altogether 4,320 children and adolescents aged 3, 6, 9, 12, 15 and 18 years were randomly chosen from the national population register of these areas. Of those invited, 3,596 participated in the cross-sectional study in 1980\(^1\). In 2001, we re-examined 2,265 of these individuals, now aged 24 to 39 years\(^2\). The study was approved by local Ethics committees and all subjects gave their written informed consent.

Clinical characteristics and risk factors

Height and weight were measured, and body mass index (BMI) was calculated. Waist and hip circumferences were measured with an accuracy of 0.1 cm. In 1980, blood pressure was measured from 3-year-olds with an ultrasound device (Arteriosonde 1020, Roche) and in others with a standard mercury sphygmomanometer. In 2001, a random zero sphygmomanometer was used. Average of three measurements was used in the analysis. In adulthood, family history of premature CHD was assessed by a questionnaire. For the determination of serum lipoprotein levels, venous blood samples were drawn after an overnight fast. All lipid and apolipoprotein determinations were done using standard methods. Lipoprotein (a) was measured in 1986 in 1,722 subjects by radioimmunoassay (Pharmacia Diagnostics, Uppsala,
Sweden). The fasting plasma high sensitive C-reactive protein concentrations (in 2001) were analyzed by latex turbidometric immunoassay (Wako Chemicals GmbH, Neuss, Germany). In 1980, serum insulin was measured using a modification of the immunoassay method of Herbert et al.\(^3\). In 2001, serum insulin was measured by microparticle enzyme immunoassay kit (Abbott Laboratories, Diagnostic Division, Dainabot). Glucose concentrations (in 2001) were analyzed enzymatically (Olympus Diagnostica GmbH, Hamburg, Germany), and homocysteine concentrations (in 2001) with microparticle enzyme immunoassay kit (Imx assay, Abbott Laboratories, Tokyo, Japan). Homeostasis model assessment (HOMA)-index was calculated from the formula: HOMA= fasting glucose [mmol/L] \(\times\) fasting insulin [\(\mu U/mL\)] / 22.5. Birth weight, socioeconomic status (number of parental school years in 1980, number of own school years in 2001), alcohol use, smoking, physical activity and diet (butter use, including butter based mixtures, and daily use of vegetables) were acquired using questionnaires. Physical activity index was constructed by combining the information on the frequency, intensity and duration of physical activity, including leisure-time physical activity and commuting to the work place. Details of methods have been presented elsewhere\(^2,4,5\)

**Ultrasound imaging**

Carotid ultrasound studies were performed in 2001 for 2,265 subjects using Sequoia 512 ultrasound mainframes (Acuson, Mountain View, CA, USA) with 13.0 MHz linear array transducer, as previously described\(^4\). Left carotid artery was scanned by ultrasound technicians following a standardized protocol. The image was focused on the posterior (far) wall and gain settings were used to optimize image quality. A resolution box function (zoom) was used to record an image of 25 mm in width and
Magnified image was recorded from the angle showing the greatest distance between the lumen-intima interface and the media-adventitia interface. A moving scan with duration of 5 seconds which included the beginning of the carotid bifurcation and the common carotid artery was recorded and stored in digital format on optical discs for subsequent off-line analysis. From the 5 second clip image, the best quality end-diastolic frame was selected (incident with the R-wave on a continuously recorded ECG). From this image, at least four measurements of the common carotid far wall were taken approximately 10 mm proximal to the bifurcation to derive maximal carotid IMT. The between-visit (2 visits 3 months apart) coefficient of variation (CV) of IMT measurements was 6.4%.

To assess carotid artery compliance (CAC), the best quality cardiac cycle was selected from the 5-second clip images. The common carotid diameter was measured at least twice in end-diastole and end-systole, respectively. The mean of the measurements was used as the end-diastolic and the end-systolic diameter. Ultrasound and concomitant brachial blood pressure measurements were used to calculate carotid artery compliance = \((D_s - D_d)/D_d)/(P_s - P_d)\), where \(D_d\) is the diastolic diameter; \(D_s\), the systolic diameter; \(P_s\), systolic blood pressure and \(P_d\), diastolic blood pressure. The between-visit coefficient of variation was 2.7% for diastolic carotid diameter and 16.3% for CAC.

Brachial artery ultrasound studies were performed successfully for 2,109 subjects, as previously reported. To assess brachial FMD, the left brachial artery diameter was measured both at rest and during reactive hyperemia. Increased flow was induced by inflation of a pneumatic tourniquet placed around the forearm to a pressure of 250 mmHg for 45 minutes, followed by a release. Three measurements of arterial diameter were performed at end-diastole at a fixed distance from an anatomic
marker at rest and 40, 60 and 80 seconds after cuff release. The vessel diameter in
scans after reactive hyperemia was expressed as the percentage relative to resting scan
(100 percent). The average of three measurements at each time point was used to
derive the maximum FMD (the greatest value between 40 to 80 seconds). The 3-
month between-visit CV was 3.2% for brachial artery diameter measurements, and
26.0% for FMD measurements. All ultrasound scans were analyzed by a single reader
blinded to subject’s details.

Statistical methods

Family history was assessed using three different classifications listed below from
stringent to broad:

Family history was considered positive only if either study subjects’ father or mother
had

A) suffered from myocardial infarction, or if either of them have had percutaneous
coronary intervention or coronary by-pass surgery at or before the age of 55 years
(N=201).

B) been diagnosed with CHD, suffered from myocardial infarction, or if either of
them have had percutaneous coronary intervention or coronary by-pass surgery at or
before the age of 55 years (N=291).

C) been diagnosed with CHD, suffered from myocardial infarction, or if either of
them have had percutaneous coronary intervention or coronary by-pass surgery at any
age (N=539).

Results were essentially similar, when using any of these 3 classifications. Unless
stated otherwise, results are expressed using classification (B).
Group comparisons were performed using t-test for continuous variables and χ²-test for categorical variables. As there was no interaction between sexes in risk variables or ultrasound variables, P-values were calculated sexes combined taking the sex difference into account by including the main effect of the sex into the regression models. Subjects with positive family history were older than those with negative history. Therefore, variables with significant differences between the groups in t-test, were also studied with regression analysis adjusted for age. To study whether the difference in ultrasound variables was independent of current risk factors and childhood risk factors identified 21 years earlier, we used linear regression models. In these analyses, all the categorical risk factors were dichotomous (dummy) variables.

To test whether risk factors have similar influence on IMT in subjects with positive or negative family history, we calculated interaction terms between subjects with positive and negative family history for the associations of risk factors and IMT. In separate models for each risk factor, we included IMT as the dependent variable and age, sex, family risk, risk factor and risk factor*family risk interaction term as the independent variables. Thereafter all significant main effects and interactions were analyzed by formulating a model in which all of them were included. The final model was constructed step by step, leaving the most non-significant term out of the model and fitting a simpler model. The final model consisted only the significant terms, and individual variables included in the interaction term.

Metabolic syndrome was defined using the National Institute of Health Adult Treatment Panel III (NCEP) criterions⁸: waist over 102 cm in men and over 88 cm in women, serum triglycerides over 1.7 mmol/l (150 mg/dl), HDL cholesterol less than 1.04 mmol/l (40 mg/dl) in men and 1.29 mmol/l (50 mg/dl) in women, blood pressure over 130 or 85 mmHg or treated, and plasma glucose over 6.1 mmol/l (110 mg/dl).
Values for triglycerides, insulin and C-reactive protein were log_{10}-transformed prior to analyses due to skewed distributions. The statistical tests were performed with Statistical Analysis System version 8.1, and statistical significance was inferred at a 2-tailed P-value <0.05.
References


