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# Life-course risk factor levels and coronary artery calcification. The Cardiovascular Risk in Young Finns Study

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ABSTRACT

*Background:* Risk factors measured in early life have been shown to predict coronary artery calcium (CAC) in adulthood. However, limited data exist on when risk factor profiles of those who develop CAC diverge from those who do not. We investigated the associations of coronary heart disease risk factor trajectories beginning in adolescence and CAC measured at middle-age.

*Methods:* CAC was measured among 589 participants aged 39–45 years in whom cardiovascular risk factors (serum lipids, blood pressure, body mass index, physical activity, smoking habits, and fruit, vegetable, fish, and butter intake) had been collected in 1980, 1983, 1986, 2001, and 2007 as part of the Cardiovascular Risk in Young Finns Study.

*Results:* Mean levels of low-density lipoprotein cholesterol (LDL-C), total cholesterol, apolipoprotein B (Apo-B), and systolic blood pressure (SBP) levels across the 27-year period were significantly higher among those with CAC vs. those without. The difference between the groups was 0.25 mmol/l (95% confidence interval, 95%CI, 0.079–0.41) for LDL-C, 0.26 mmol/l (95%CI 0.080–0.44) for total cholesterol, 0.05 mmol/l (95%CI 0.0085–0.091) for Apo-B and 1.92 mmHg (95%CI 0.10–3.74) for SBP after adjustment for other risk factors. Those with CAC at age 39–45 years had higher serum lipid levels already in adolescence or early adulthood compared with those without CAC, with these differences becoming more pronounced during the life-course.

*Conclusions:* Long-time risk factor exposure to higher LDL-C, total cholesterol and Apo-B levels already starting in adolescence and higher SBP levels in adulthood is associated with CAC at middle-age.



## 1. Introduction

Treating established coronary heart disease (CHD) in late adulthood may be *"locking the barn door after the horse has been stolen"* [1]. Evidence from autopsy [2,3] and large-scale epidemiological imaging studies [4–10] not only supports the origin of atherosclerotic CHD to early life, but that the same risk factors shown to predict adult CHD are operating already in young persons. Quantified non-invasively using cardiac computed tomography, coronary artery calcification (CAC) is an intermediate end-point for CHD that correlates proportionally with the extent and severity of coronary atherosclerosis [11].

Data from the Muscatine, CARDIA, and Young Finns cohort studies have shown direct and independent effects of early-life (extending to adolescence) risk factors measured at a single time-point with CAC in adulthood [4,7,12]. However, long-term trials that are able to provide information on the potential benefits of risk factor modification that

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span youth and adulthood on later development of CAC are unlikely. Because of this, cohort studies with multiple measurement time-points collected across the life-course provide an opportunity to gain insight on the point in time when those who develop CAC diverge from those who do not in regard to CHD risk factors. Although one extremely important observation that has emerged from the above-mentioned cohort studies has been that risk factors measured in youth to be stronger predictors of CAC than measurements obtained at the time imaging was performed [12,13], only limited data exist on the potential effect that risk factor levels over the life-course may have on CAC. Previously a few studies have shown that higher life-course trajectories of risk factors, such as body mass index (BMI), central fatness and blood pressure, are associated with CHD [14,15] or subclinical CHD [16,17].

Therefore, we examined if long-time exposure to adverse risk factor levels is associated with development of CAC at middle-age using data from the Cardiovascular Risk in Young Finns Study.

## 2. Methods

### 2.1. Participants

The Cardiovascular Risk in Young Finns Study is an ongoing follow-up of atherosclerosis risk factors of Finnish children and young adults carried out in all five Finnish university cities with medical schools (Helsinki, Kuopio, Oulu, Tampere, and Turku) and their rural surrounds. The first cross-sectional survey was conducted in 1980 on 3596 participants aged 3–18 years. Follow-up surveys were conducted in 1983, 1986, 2001, and 2007. In 2007, 2204 individuals of the original cohort participated. In 2008, a cardiac computed tomography (CT) study to measure coronary-artery calcium (CAC) was conducted among 589 individuals, then aged 40–46 years. The CT study was offered to all 802 participants from the 3 oldest cohorts who resided in the 3 centers (Turku, Tampere and Kuopio) with CAC imaging capability. The attendance rate was 73% (Supplementary Fig. 1). Risk factor levels were available for 589 subjects in 1980, 476 in 1983, 407 in 1986, 505 in 2001 and 539 in 2007. Full details of the methods have been described previously [13]. Participants gave written informed consent and the study was approved by local ethics committees.

### 2.2. Computed tomography imaging

The CT scans were performed with a GE Discovery VCT 64-slice CT/PET device (Turku), a Philips Brilliance 64-slice CT device (Tampere) and a Siemens Somaton Sensation 16-slice CT device (Kuopio). CAC scores were calculated using the Agatston method for each coronary artery [18]. The coefficient of variation for intraobserver measurements was 4.0%. A phantom with deposits of known calcium concentration was also scanned twice using 3 projections at all of the study centers and the coefficient of variation between all of the phantom scans was 3.9%. Presence of CAC was defined as an Agatston score of 1 or greater. Full details of the study protocol have been published previously [13].

### 2.3. Clinical and biochemical characteristics

BMI was calculated as weight, kg/(height, m)$^2$. Blood pressure was measured using a standard mercury sphygmomanometer in 1980 and a random zero sphygmomanometer in 1983, 1986, 2001 and 2007. Smoking habits, vegetable, fruit, and fish consumption, type of spread usually used on bread (butter vs. margarine) and physical activity were inquired with a questionnaire. Pack years of smoking were calculated as the number of cigarette packs smoked daily multiplied by the duration of daily smoking in years. Physical activity index was calculated as a metabolic equivalent index by assessing the duration, intensity, and frequency of physical activity, including leisure-time physical activity and commuting [19].

Venous blood samples were taken after an overnight fast. Standard methods were used to determine serum lipids, glucose, and insulin concentrations. Details of the methods have been described previously [20]. Glucose measurement was not performed in 1980 or 1983. Computed values for apolipoprotein A-1 (Apo-A1) and B (Apo-B) were created for 1980, 1983, and 1986, as described previously [21]. Low-density lipoprotein cholesterol (LDL-C) was calculated indirectly using the Friedewald formula [20]. In 1980, 1983, and 1986, serum insulin was measured using a modification of the immunoassay method and in 2001 and 2007, by microparticle enzyme immunoassay kit.

### 2.4. Statistical analysis

We compared the risk factor trajectories (from years 1980, 1983, 1986, 2001 and 2007) using multi-level mixed modeling with maximum-likelihood estimation as a function of age for two groups; those with CAC (Agatston score 1 or above) in 2008 and those without CAC (Agatston score 0). This approach allows for missing data (assuming missing data are random) and takes into account correlations between repeated measures on the same individual. On average, subjects attended 4.3 measurements (range 1–5, SD = 0.94). We fitted interaction terms between CAC group (0 vs 1) and time to compare the trajectory of risk factors between CAC groups, allowing us to determine the age at which differences in risk factor levels were apparent. A global test for parallelism of trajectories was conducted to study overall longitudinal differences between risk factor levels.

The models were first fitted with sex as a dichotomous variable and time as a multichotomous variable (model 1) and then additional risk factors; physical activity index as a multichotomous variable, pack years of smoking and vegetable, fruit, and fish consumption (model 2), BMI (model 3), systolic blood pressure (SBP) (model 4A) and LDL-C (model 4B) as continuous variables. A compound symmetry covariance structure, which assumes a constant correlation between separate measurements, was used in all models. Least square means were calculated for the risk factor levels by CAC group, both at specific age-points as well as a longitudinal mean value throughout the study period. To study the effects of risk factor levels on CAC severity, we repeated the analyses with three CAC groups: no CAC, CAC score 1–99 and CAC score ≥100. Insulin levels, which were skewed, were log-transformed before all analyses. A P-value of $<0.05$ was considered statistically significant. All computations were performed using SAS version 9.4 or STATA version 13.1.

## 3. Results

The mean age of participants at follow-up was 41.7 ± 2.4 years and 44.5% were male. The prevalence of CAC was 19.2% (12.2% for women, 27.9% for men). Total CAC scores of 1–100, 101–400 and >400 were measured in 16.7%, 1.9% and 0.7% of the subjects, respectively. Seventy participants were either on antihypertensive (N = 62), or lipid-lowering medication (N = 19), oral medication for diabetes (N = 2), or used injected insulin (N = 4). No substantial difference in results was observed after these participants were excluded from the analyses (data not shown).

A comparison of follow-up characteristics between participants and non-participants within the three oldest cohorts was conducted to study the representativeness of the participating study population (Supplementary Table 1). No significant differences between the groups were found.

General characteristics of the study population at each age point stratified by CAC status at follow-up are shown in Table 1. Mean risk factor levels by CAC status at follow-up are shown in Table 2. On average, subjects with CAC at age 39–45 years had greater mean levels of serum cholesterol (0.29 mmol/l; 95%CI 0.13–0.46), LDL-C (0.27 mmol/l; 95%CI 0.11–0.42), Apo-B (0.062 g/l, 95%CI 0.022–0.10), and SBP (2.25 mmHg, 95%CI 0.39–4.11) across the life-course compared with those without CAC. Adjustment for lifestyle variables (model 2), BMI (model 3), SBP (model 4A) or LDL-C (model 4B) did not alter the results substantially (Table 3). Levels of HDL-C, triglycerides, Apo-A1, glucose, insulin, BMI, physical activity index, diastolic blood pressure (DBP), and consumption of vegetables, fruit, fish, and butter as well as prevalence of daily smoking were not significantly different across the CAC groups.

General characteristics at each age point and mean risk factor levels by CAC severity are shown in Supplementary Tables 2 and 3, respectively. An increasing trend in mean levels of LDL-C, serum cholesterol and Apo-B, but not SBP, was seen across CAC severity. The difference between subjects with no CAC vs a CAC score ≥100 was 0.48 mmol/l (95%CI 0.11–0.85; P = 0.001) for LDL-C, 0.44 mmol/l (95%CI 0.04–0.85, P = 0.002) for serum cholesterol and 0.12 g/l; (95%CI 0.02–0.21, P = 0.004) for Apo-B.

Trajectories for risk factors are shown in Fig. 1 and Supplementary Fig. 2. The difference in risk factor levels between CAC groups was statistically significant already in adolescence (age 15) for LDL-C and early adulthood for total cholesterol and Apo-B (age 21). These gaps were amplified during the life-course (0.21 mmol/l at age 15 vs. 0.54 mmol/l at age 42 for LDL-C, 0.27 mmol/l at age 21 vs. 0.63 mmol/l for total cholesterol and 0.07 mmol/l at age 21 vs. 0.15 mmol/l at age 42 for Apo-B). A significant difference between the CAC groups was also seen for DBP at 33 and for SBP at 39 years of age increasing from 5.1 to 6.1 mmHg and from 3.5 to 5.2 mmHg, respectively by age 45 years. An increase in serum cholesterol, LDL-C, and Apo-B, and a decrease in HDL-C and SBP levels was seen between ages 24–33 for both groups.

**Table 1**
Mean (±SE) risk factor levels of the study population across the 28-year follow-up period by CAC status at age 39–45 years.

| Age | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Characteristics | Group | 12 | 15 | 18 | 21 | 24 | 33 | 36 | 39 | 42 | 45 |
| LDL-C (mmol/l) | No CAC | 3.5 ± 0.05 | 3.1 ± 0.04 | 3.0 ± 0.04 | 3.0 ± 0.05 | 2.9 ± 0.07 | 3.4 ± 0.06 | 3.4 ± 0.06 | 3.2 ± 0.05 | 3.2 ± 0.06 | 3.1 ± 0.06 |
| | CAC | 3.7 ± 0.1 | 3.3 ± 0.1 | 3.3 ± 0.09 | 3.3 ± 0.1 | 3.1 ± 0.1 | 3.6 ± 0.1 | 3.9 ± 0.1 | 3.6 ± 0.09 | 3.7 ± 0.1 | 3.1 ± 0.1 |
| HDL-C (mmol/l) | No CAC | 1.6 ± 0.02 | 1.6 ± 0.02 | 1.5 ± 0.01 | 1.6 ± 0.02 | 1.5 ± 0.03 | 1.3 ± 0.02 | 1.3 ± 0.02 | 1.3 ± 0.02 | 1.3 ± 0.02 | 1.3 ± 0.02 |
| | CAC | 1.6 ± 0.04 | 1.6 ± 0.03 | 1.5 ± 0.03 | 1.6 ± 0.04 | 1.5 ± 0.05 | 1.3 ± 0.05 | 1.3 ± 0.05 | 1.3 ± 0.03 | 1.3 ± 0.05 | 1.4 ± 0.04 |
| Total cholesterol (mmol/l) | No CAC | 5.4 ± 0.06 | 5.0 ± 0.05 | 5.0 ± 0.05 | 5.1 ± 0.06 | 5.0 ± 0.08 | 5.3 ± 0.07 | 5.3 ± 0.07 | 5.2 ± 0.05 | 5.2 ± 0.07 | 5.1 ± 0.07 |
| | CAC | 5.6 ± 0.1 | 5.2 ± 0.1 | 5.2 ± 0.1 | 5.4 ± 0.1 | 5.2 ± 0.1 | 5.6 ± 0.1 | 5.8 ± 0.1 | 5.5 ± 0.1 | 5.8 ± 0.1 | 5.2 ± 0.1 |
| Triglycerides (mmol/l) | No CAC | 0.6 ± 0.05 | 0.8 ± 0.04 | 1.0 ± 0.04 | 1.1 ± 0.05 | 1.1 ± 0.07 | 1.3 ± 0.06 | 1.4 ± 0.06 | 1.5 ± 0.04 | 1.5 ± 0.06 | 1.5 ± 0.06 |
| | CAC | 0.7 ± 0.1 | 0.7 ± 0.09 | 0.8 ± 0.08 | 1.2 ± 0.09 | 1.2 ± 0.1 | 1.5 ± 0.1 | 1.4 ± 0.1 | 1.5 ± 0.09 | 1.7 ± 0.1 | 1.5 ± 0.1 |
| Apo-A1 (g/l) | No CAC | 1.6 ± 0.02 | 1.6 ± 0.01 | 1.6 ± 0.01 | 1.7 ± 0.01 | 1.6 ± 0.02 | 1.5 ± 0.02 | 1.5 ± 0.02 | 1.5 ± 0.01 | 1.6 ± 0.02 | 1.6 ± 0.02 |
| | CAC | 1.6 ± 0.03 | 1.6 ± 0.03 | 1.6 ± 0.02 | 1.7 ± 0.03 | 1.6 ± 0.04 | 1.5 ± 0.04 | 1.5 ± 0.04 | 1.6 ± 0.03 | 1.6 ± 0.04 | 1.7 ± 0.03 |
| Apo-B (g/l) | No CAC | 1.0 ± 0.02 | 0.9 ± 0.01 | 0.9 ± 0.01 | 1.0 ± 0.02 | 0.9 ± 0.02 | 1.1 ± 0.02 | 1.1 ± 0.02 | 1.1 ± 0.01 | 1.1 ± 0.02 | 1.1 ± 0.02 |
| | CAC | 1.0 ± 0.04 | 0.9 ± 0.03 | 0.9 ± 0.02 | 1.0 ± 0.03 | 1.0 ± 0.04 | 1.2 ± 0.04 | 1.2 ± 0.04 | 1.2 ± 0.03 | 1.2 ± 0.04 | 1.1 ± 0.03 |
| Non-HDL-C (mmol/l) | No CAC | 3.8 ± 0.06 | 3.5 ± 0.05 | 3.5 ± 0.05 | 3.5 ± 0.06 | 3.4 ± 0.08 | 4.0 ± 0.06 | 4.0 ± 0.07 | 3.9 ± 0.05 | 3.8 ± 0.07 | 3.8 ± 0.07 |
| | CAC | 4.0 ± 0.1 | 3.7 ± 0.1 | 3.7 ± 0.09 | 3.8 ± 0.1 | 3.6 ± 0.1 | 4.3 ± 0.1 | 4.5 ± 0.1 | 4.3 ± 0.1 | 4.5 ± 0.1 | 3.8 ± 0.1 |
| Glucose (mmol/l) | No CAC | na | na | 4.6 ± 0.06 | 4.7 ± 0.07 | 4.6 ± 0.06 | 5.0 ± 0.05 | 5.1 ± 0.06 | 5.3 ± 0.04 | 5.4 ± 0.05 | 5.6 ± 0.06 |
| | CAC | na | na | 4.7 ± 0.1 | 4.5 ± 0.1 | 4.7 ± 0.1 | 5.3 ± 0.1 | 5.2 ± 0.1 | 5.5 ± 0.08 | 5.4 ± 0.1 | 5.7 ± 0.1 |
| Insulin (mU/l) | No CAC | 12.3 ± 0.5 | 13.7 ± 0.4 | 12.1 ± 0.3 | 10.2 ± 0.5 | 9.4 ± 0.7 | 7.5 ± 0.5 | 7.5 ± 0.5 | 7.8 ± 0.4 | 8.8 ± 0.5 | 10.0 ± 0.6 |
| | CAC | 11.0 ± 1.1 | 14.1 ± 0.8 | 13.0 ± 0.7 | 11.1 ± 0.9 | 8.4 ± 1.2 | 7.9 ± 1.2 | 8.3 ± 1.2 | 9.1 ± 0.8 | 10.4 ± 1.2 | 14.8 ± 1.0 |
| BMI (kg/m$^2$) | No CAC | 17.9 ± 0.2 | 20.0 ± 0.2 | 21.5 ± 0.2 | 22.2 ± 0.2 | 22.9 ± 0.3 | 25.4 ± 0.3 | 25.6 ± 0.3 | 26.7 ± 0.2 | 26.5 ± 0.3 | 27.4 ± 0.3 |
| | CAC | 18.4 ± 0.5 | 20.0 ± 0.4 | 21.6 ± 0.4 | 23.0 ± 0.4 | 23.2 ± 0.5 | 26.2 ± 0.6 | 26.4 ± 0.5 | 26.9 ± 0.4 | 27.6 ± 0.5 | 27.5 ± 0.5 |
| Physical activity index | No CAC | 9.5 ± 0.1 | 8.8 ± 0.1 | 8.3 ± 0.1 | 8.1 ± 0.1 | 7.9 ± 0.2 | 9.9 ± 0.2 | 9.1 ± 0.2 | 9.1 ± 0.1 | 8.6 ± 0.2 | 8.6 ± 0.2 |
| | CAC | 9.4 ± 0.3 | 9.3 ± 0.2 | 8.6 ± 0.2 | 8.0 ± 0.2 | 7.7 ± 0.4 | 9.5 ± 0.3 | 9.4 ± 0.3 | 9.0 ± 0.2 | 8.9 ± 0.3 | 8.5 ± 0.3 |
| SBP (mm Hg) | No CAC | 112 ± 0.8 | 118 ± 0.6 | 121 ± 0.6 | 124 ± 0.8 | 123 ± 1.1 | 118 ± 0.9 | 118 ± 0.9 | 121 ± 0.7 | 123 ± 0.9 | 125 ± 1.0 |
| | CAC | 112 ± 1.9 | 116 ± 1.4 | 123 ± 1.2 | 125 ± 1.5 | 126 ± 2.0 | 121 ± 2.0 | 120 ± 2.0 | 124 ± 1.4 | 127 ± 2.0 | 130 ± 1.7 |
| DBP (mm Hg) | No CAC | 70 ± 0.7 | 68 ± 0.6 | 70 ± 0.5 | 71 ± 0.7 | 71 ± 1.0 | 72 ± 0.8 | 73 ± 0.8 | 75 ± 0.6 | 77 ± 0.8 | 77 ± 0.9 |
| | CAC | 70 ± 1.7 | 66 ± 1.2 | 70 ± 1.1 | 68 ± 1.3 | 71 ± 1.7 | 77 ± 1.8 | 73 ± 1.8 | 78 ± 1.2 | 79 ± 1.7 | 83 ± 1.5 |
| Smoking prevalence (%) | No CAC | 0 | 15.0 | 20.7 | 14.9 | 17.8 | 24.4 | 17.6 | 17.7 | 16.7 | 12.0 |
| | CAC | 0 | 9.5 | 21.3 | 18.8 | 10.0 | 31.3 | 22.6 | 23.6 | 16.1 | 22.0 |
| Vegetable servings/week | No CAC | 6.1 ± 0.2 | 5.5 ± 0.2 | 5.4 ± 0.2 | 5.9 ± 0.2 | 6.0 ± 0.3 | 5.4 ± 0.2 | 5.8 ± 0.2 | 6.0 ± 0.3 | na | na |
| | CAC | 5.9 ± 0.5 | 5.35 ± 0.4 | 4.91 ± 0.3 | 5.81 ± 0.4 | 5.01 ± 0.5 | 4.98 ± 0.5 | 4.86 ± 0.5 | 6.5 ± 0.5 | na | na |
| Fruit servings/week | No CAC | 6.8 ± 0.2 | 6.1 ± 0.2 | 5.9 ± 0.2 | 5.9 ± 0.2 | 6.3 ± 0.3 | 5.2 ± 0.2 | 6.2 ± 0.2 | 5.5 ± 0.3 | na | na |
| | CAC | 7.3 ± 0.2 | 5.9 ± 0.4 | 5.4 ± 0.3 | 5.9 ± 0.4 | 5.7 ± 0.5 | 6.1 ± 0.5 | 5.7 ± 0.5 | 6.3 ± 0.5 | na | na |
| Use of butter (%) | No CAC | 67.7 | 71.5 | 68.2 | 60.6 | 58.0 | 26.6 | 29.0 | 21.3 | 9.4 | 9.5 |
| | CAC | 73.6 | 80.5 | 73.5 | 69.6 | 64.6 | 25.0 | 32.0 | 23.1 | 11.9 | 15.2 |
| Fish servings/week | No CAC | 1.1 ± 0.09 | 1.0 ± 0.06 | 1.0 ± 0.06 | 1.2 ± 0.08 | 1.0 ± 0.1 | 1.0 ± 0.09 | 1.0 ± 0.09 | 1.4 ± 0.1 | na | na |
| | CAC | 1.0 ± 0.2 | 1.0 ± 0.1 | 1.0 ± 0.1 | 1.0 ± 0.1 | 1.1 ± 0.2 | 0.8 ± 0.2 | 0.7 ± 0.2 | 1.3 ± 0.2 | na | na |

## 4. Discussion

We investigated the associations of life-time CHD risk factors and CAC at middle-age and found that mean longitudinal values for LDL-C, total cholesterol, Apo-B, and SBP were higher among those with CAC at follow-up compared with those without and that these risk factors were independently associated with CAC in multivariable analyses. For LDL-C, total cholesterol, and Apo-B, a significant difference between the groups was present already in adolescence or early adulthood with the difference becoming more pronounced with age, whereas for SBP, only adult levels were significantly higher for those with CAC. Levels of LDL-C, total cholesterol and Apo-B were also associated with the severity of CAC.

To our knowledge, no previous studies have used the life-course analysis approach to examine the effect of risk factors across childhood, adolescence, early and mid-adulthood on CAC. Previously, Allen et al. distinguished 5 blood pressure trajectories in the CARDIA study and found that those with stable moderate, moderate-increasing, stable high and high-increasing trajectories were at an increasing risk of developing CAC at middle age [16]. Ferreira et al. demonstrated that persistent central fatness and elevated blood pressure were associated with stiffer carotid arteries at follow-up 24 years later in the Amsterdam Growth and Health Longitudinal Study [17]. In a large study conducted through the Staff Periodic Examination Center of the Israeli Army Medical Corps, the risk of angiography-proven CHD was associated with an elevated BMI both in adolescence and adulthood [14]. Reinikainen et al. found that cumulative risk factor levels, including SBP and total cholesterol, predicted cardiovascular disease mortality in a 50-year follow-up study better than a model using only the most recent measurements [15]. Our results support the findings in these studies in that differences in risk factor levels between subjects with versus those without atherosclerotic changes in their vasculature are present early in life, even adolescence, and become larger in adulthood. The main implication of these studies is that maintaining lower risk factor

**Table 2**
Mean (±SE) risk factor levels from 1980, 1983, 1986, 2001, and 2007 by CAC status in 2008.

| Risk factor | No CAC (N = 476) | CAC present (N = 113) | P-value[4] |
|---|---|---|---|
| Male sex, % | 39.7 | 64.6 | <0.0001 |
| LDL-C, mmol/l | 3.20 ± 0.03 | 3.46 ± 0.07 | 0.0006 |
| HDL-C, mmol/l | 1.43 ± 0.01 | 1.43 ± 0.02 | 0.90 |
| Total cholesterol, mmol/l | 5.15 ± 0.04 | 5.45 ± 0.08 | 0.0006 |
| Triglycerides, mmol/l | 1.19 ± 0.02 | 1.23 ± 0.05 | 0.42 |
| Apo-A1[1], g/l | 1.58 ± 0.009 | 1.60 ± 0.02 | 0.42 |
| Apo-B[1], g/l | 1.01 ± 0.009 | 1.07 ± 0.02 | 0.002 |
| Glucose[2], mmol/l | 5.1 ± 0.03 | 5.1 ± 0.05 | 0.30 |
| Insulin, mU/l | 9.9 ± 0.2 | 10.9 ± 0.4 | 0.28 |
| BMI, kg/m$^2$ | 23.6 ± 0.15 | 24.1 ± 0.3 | 0.17 |
| Physical activity index | 8.8 ± 0.07 | 8.8 ± 0.1 | 0.84 |
| SBP, mm Hg | 120.3 ± 0.4 | 122.5 ± 0.8 | 0.02 |
| DBP, mm Hg | 72.3 ± 0.3 | 73.6 ± 0.7 | 0.12 |
| Daily smoking, % | 15.9 | 17.7 | 0.61 |
| Vegetable servings/week[3] | 5.7 ± 0.1 | 5.5 ± 0.2 | 0.44 |
| Fruit servings/week[3] | 6.0 ± 0.1 | 6.0 ± 0.2 | 0.87 |
| Use of butter (%) | 42.1 | 46.9 | 0.11 |
| Fish servings/week[3] | 1.1 ± 0.04 | 1.0 ± 0.07 | 0.19 |

[1] Estimated values for Apo-A1 and Apo-B used for years 1980, 1983, and 1986.
[2] Measurements available for 1986, 2001 and 2007.
[3] Information not available for 2007.
[4] Adjusted for age and sex, where applicable.

**Table 3**
Mean differences in risk factor levels throughout the 27-year study period between subjects with vs. without CAC at age 42–45 years.

| | | LDL-C (mmol/l) | | Total cholesterol (mmol/l) | | Apo-B (g/l) | | SBP (mm Hg) | |
|---|---|---|---|---|---|---|---|---|---|
| Model | Adjustments | β[1] | 95% CI | β | 95% CI | β | 95% CI | β | 95% CI |
| 1 | Sex, time | 0.27‡ | 0.11–0.42 | 0.29‡ | 0.13–0.46 | 0.062† | 0.022–0.10 | 2.25* | 0.39–4.11 |
| 2 | Model 1 + PAI, PY, vegetable, fruit, and fish consumption | 0.26† | 0.091–0.42 | 0.28† | 0.97–0.46 | 0.060† | 0.018–0.10 | 2.38* | 0.47–4.29 |
| 3 | Model 2 + BMI | 0.24† | 0.071–0.40 | 0.25† | 0.0073–0.44 | 0.049* | 0.0077–0.091 | 1.83* | 0.010–3.64 |
| 4 A | Model 3 + SBP | 0.24† | 0.079–0.41 | 0.26† | 0.079–0.44 | 0.049† | 0.0080–0.091 | … | … |
| 4B | Model 3 + LDL-C | … | … | … | … | … | … | 1.92* | 0.097–3.74 |

*P < 0.05, †P < 0.01, ‡P < 0.001.
Abbreviations: PAI = physical activity index, PY = pack years of smoking, BMI = body-mass index, SBP = systolic blood pressure, LDL-C = low-density lipoprotein cholesterol.
[1] The β-values are longitudinal regression coefficients that indicate mean differences in risk factor levels across the follow-up period between subjects with CAC vs. those without in 2008.

levels throughout the life-course, not only in middle-age, is important in the prevention of CHD.

Several studies have shown the associations of childhood, adolescence or early adulthood risk factor levels with adult subclinical CHD identified by either CAC or carotid artery measurements by ultrasound [4–10]. This study adds to the growing data base that life-time risk factor exposure starting already in adolescence plays an important role in the pathogenesis of CHD. In our study, LDL-C levels were higher among those with CAC at follow-up already at age 15, indicating that a vulnerable period in the development of CAC takes place in adolescence. In addition to this, these subjects were characterized by higher LDL-C and Apo-B levels as well as sharper increases throughout their life-course and LDL-C and Apo-B that were independently associated with CAC after multivariable adjustment. High LDL-C is a well-established CHD risk factor and the rate of movement of LDL particles from plasma into the arterial wall is dependent on its plasma concentration [22], while Apo-B is the primary apolipoprotein of LDL and its childhood levels have previously been found to be associated with subclinical atherosclerosis in adulthood in both the Bogalusa [23] and the Young Finns [24] studies.

An increase in LDL-C, Apo-B, and serum cholesterol and a decrease in HDL-C and SBP levels were seen in young adulthood. This is partly due to age-related effects in risk factor levels. In addition, we have previously reported secular trends in the Young Finns study population [25,26], notably decreasing BP and HDL-C as well as LDL-C levels, which explain some of the changes seen in the study.

We also found a significant difference in the longitudinal mean values of SBP between CAC groups, which is in line with our previous finding that adolescence SBP is associated with CAC regardless of change in the 27-year follow-up [13]. However, in this study utilizing more measurements, significant differences between CAC at separate age points groups were seen only in adulthood. Juhola et al. found that the effect of elevated childhood BP on carotid atherosclerosis was significantly reduced when BP levels were normalized in adulthood compared to those with persistently high BP or whose BP levels became high in adulthood using data from the Young Finns, Bogalusa, CDAH and Muscatine studies [27]. In the CARDIA study, subjects with the greatest exposure to high blood pressure levels, i.e. elevated levels in young adulthood as well as middle-age, had a significantly higher risk of developing CAC than those with stable low BP levels. A resolution group (high BP levels in young adulthood and normal levels in adulthood) was not identified in the CARDIA study. Together these data suggest that although BP levels in the early life are associated with subclinical atherosclerosis, their effects are not entirely irreversible.

### 4.1. Limitations

This study has some limitations. Because of the relatively young age of the subjects, CAC as a marker of subclinical CHD, rather than CHD events, was used as an end-point. However, studies have shown CAC to be strongly associated with subsequent symptomatic CHD [11]. In addition, we did not have measured Apo-A1 and Apo-B values for most participants 1980 and 1983 and therefore used computationally estimated values derived from serum total cholesterol, HDL-cholesterol and triglycerides, which may have affected analyses utilizing these risk factors. However, according to our previous study, correlations between estimated and measured Apo-B and Apo-A1 were strong and these correlations were replicated in over 15,000 Caucasian subjects [21]. Owing to the longitudinal design of this study, bias due to differential loss to follow-up is a potential concern. However, we have previously shown those lost to follow-up do not differ with respect to the major risk factors at baseline [20] and attrition in our study has been substantially less when compared to other similar studies [28]. Moreover, we found no differences between the subset that participated at clinics in 2007 but did not attend screenings for CAC and those that attended both the study clinics and CAC screenings. Collectively therefore, we expect bias due to differential loss to follow-up to be minimal. In addition, we lacked data on specific dietary nutrients, which may influence cardiovascular calcification, such as calcium, phosphorous, magnesium and vitamin D [29]. Our negative results should be viewed cautiously as our study population was rather young and therefore other risk factors than the ones we found may be important in the development of CAC at older ages. Additionally, results of certain age-specific trajectory analyses, especially at ages 12 and 45, may have been underpowered to distinguish differences between CAC groups due to a relatively low number of subjects.

## 5. Conclusion

In conclusion, we found that non-favorable CHD risk marker levels are already present throughout adolescence and early adulthood in subjects with CAC at follow-up. A difference in LDL-C, total cholesterol, and Apo-B levels between subjects with CAC and those without was seen already in adolescence or early adulthood and the gap in these risk factor levels was amplified in adulthood. This emphasizes the role of life-long risk factor exposure on the pathophysiology of CHD. Furthermore, identification of those at risk for developing CAC in mid adulthood might be achieved by measuring LDL-C levels already in adolescence.

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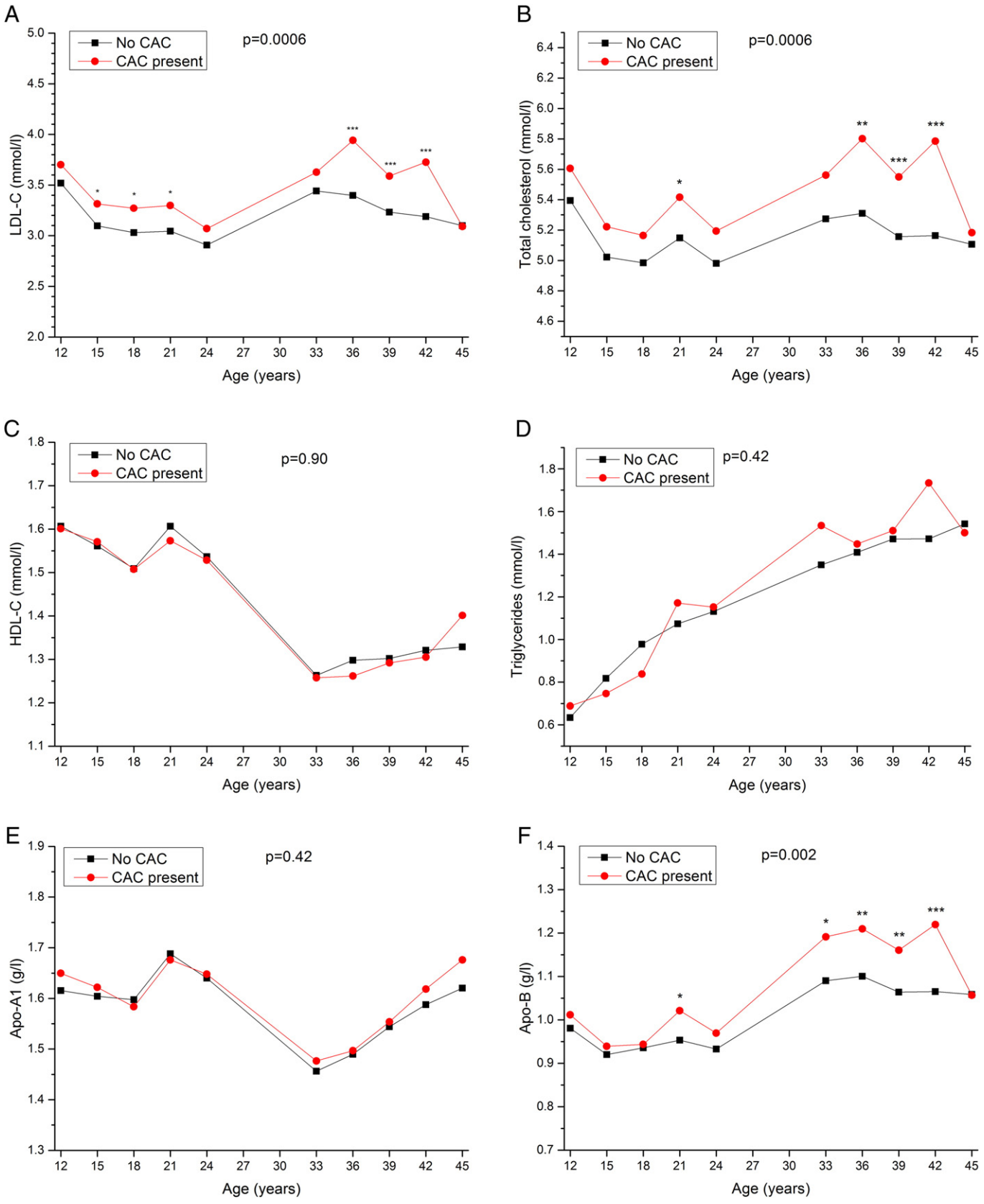


**Fig. 1.** Life-course trajectories for CHD risk factors by CAC status at follow-up: A) LDL-C; B) Total cholesterol; C) HDL-C; D) Triglycerides; E) Apo-A1; F) Apo-B; G) BMI; H) SBP; I) DBP; J) Glucose. Age-specific and global p-values from mixed model analyses adjusted for sex. *P < 0.05, **P < 0.01, ***P < 0.001.

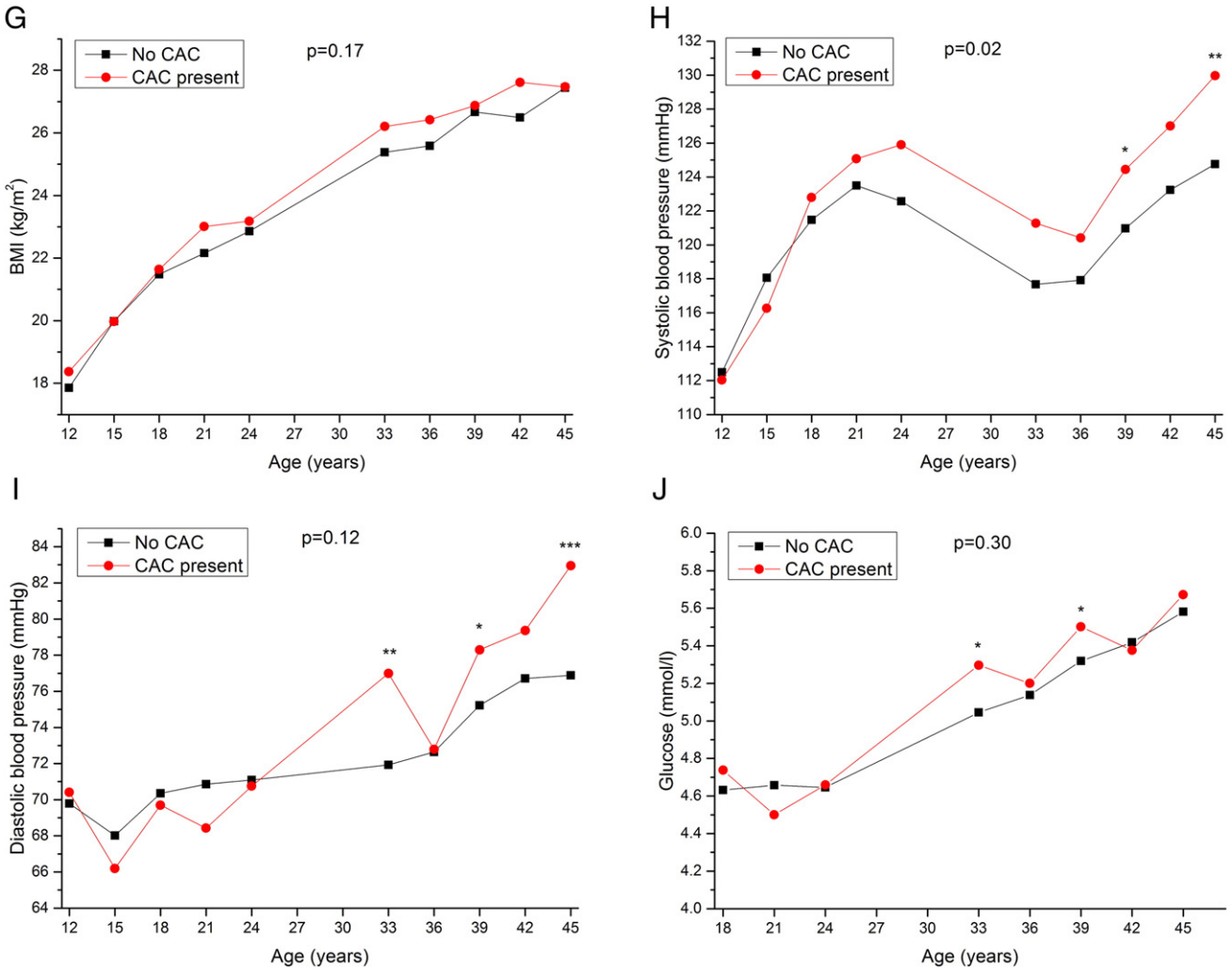


**Fig. 1** (*continued*).

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## Conflict of interest

The authors report no relationships that could be construed as a conflict of interest.

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