Atherosclerosis 263 (2017) 293–300

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

C-reactive protein in atherosclerosis – A risk marker but not a causal factor? A 13-year population-based longitudinal study: The Tromsø study

Agnethe Eltoft [a, b, *], Kjell Arne Arntzen [a, b], John-Bjarne Hansen [d, e], Tom Wilsgaard [c], Ellisiv B. Mathiesen [a, b], Stein Harald Johnsen [a, b]

[a] Department of Clinical Medicine, The Arctic University of Norway, Tromsø, Norway
[b] Department of Neurology, University Hospital of North Norway, Tromsø, Norway
[c] Department of Community Medicine, The Arctic University of Norway, Tromsø, Norway
[d] K.G. Jebsen Thrombosis Research and Expertise Center, Department of Clinical Medicine, UiT the Arctic University of Norway, Tromsø, Norway
[e] Division of Internal Medicine, University Hospital of North Norway, Tromsø, Norway

ARTICLE INFO

Article history:
Received 7 April 2017
Received in revised form
22 June 2017
Accepted 4 July 2017
Available online 5 July 2017

Keywords:
Atherosclerosis
Carotid plaque
CRP
Inflammation
Cohort study
Epidemiology
Sex-differences

ABSTRACT

Background and aims: CRP predicts cardiovascular disease (CVD) in large epidemiologic studies. The aim of the present study was to elucidate the role of CRP in atherosclerosis formation and progression in a prospective population-based study.

Methods: 6503 middle-aged subjects from The Tromsø study had serum CRP, carotid ultrasound and complete covariate data collected at baseline in 1994. Of these, 4730 and 2917 attended follow-up surveys with repeated assessments in 2001 and 2007, respectively. The cross-sectional associations between CRP and subclinical carotid atherosclerosis, and the longitudinal associations between baseline CRP and novel plaque formation and plaque progression were assessed in generalized estimating equations and linear mixed models stratified by sex.

Results: At baseline, traditional risk factors and plaque prevalence increased by CRP risk categories (<1 mg/L, 1–3 mg/L, and >3 mg/L) in both sexes. In cross-sectional analyses, multivariable-adjusted CRP was associated with plaque prevalence and total plaque area (TPA) in men and women. Age-adjusted baseline CRP >3 mg/L compared to CRP <1 mg/L predicted novel plaque formation (OR 1.44, CI 1.08–1.92) and TPA progression (β = 0.0.029 (CI, 0.003–0.056)) in men, but not in women. In neither men nor women was baseline CRP a predictor of TPA-progression or novel plaque formation when adjusted for traditional risk factors.

Conclusions: CRP was associated with plaque presence and TPA in cross-sectional analyses, but was not an independent predictor of novel plaque formation or plaque progression. Our findings suggest that CRP may link to CVD by other mechanisms than promoting formation and progression of atherosclerotic plaques.

1. Introduction

Numerous reports underline the significance of inflammation in the development, progression and destabilization of atherosclerotic lesions. The most widely investigated serum inflammatory marker in clinical practice is C-reactive protein (CRP) assessed by high-sensitivity assays. CRP has shown ability to predict cardiovascular disease (CVD) in more than 40 large epidemiological studies [1] and in a meta-analysis comprising individual participant records from 54 long-term prospective studies [2]. CRP has been included in risk assessment algorithms to discriminate subjects classified at intermediate CVD risk by traditional risk factors into higher or lower risk categories. The most recent guidelines from the American Heart Association recommend cut-off points of 2 mg/L CRP [3]. Earlier

Abbreviations: BMI, body mass index; CRP, C-reactive protein; CVD, cardiovascular disease; HDL-C, high density lipoprotein cholesterol; IQR, interquartile range; IMT, intima media thickness; TPA, total plaque area; sqrtTPA, square root transformed TPA; GEE, generalized estimating equations.

* Corresponding author. Department of Neurology, University Hospital of North Norway, 9038 Tromsø, Norway.
E-mail address: agnethe.eltoft@unn.no (A. Eltoft).

http://dx.doi.org/10.1016/j.atherosclerosis.2017.07.001
0021-9150/

proposed cut-off points of low risk (<1.0 mg/L), average risk (1.0–3.0 mg/L), and high risk (>3.0 mg/L) correspond to approximate tertiles of CRP in the adult population. Increase in relative risk estimates for CVD ranges from 1.45 to approximately 2 fold when comparing the highest with the lowest CRP tertile [4,5]. In addition to its role in risk prediction, CRP has been proposed as a tool to select patients for and tailor treatment with statins. Treatment with statins reduces both low density lipoprotein cholesterol and CRP levels. Reduction of CRP by statins is proposed to contribute to additional CVD risk reduction benefit beyond that obtained from cholesterol lowering [6].

During the last two decades, there has been an ongoing discussion about the role of CRP in atherosclerosis. Is it a true risk factor or an epiphenomenon to the atherosclerotic process? A risk factor has been defined as a factor that is "associated with a disease by virtue of its participation in the causal pathway leading to the disease" [4]. In contrast, a risk marker is statistically associated with the disease, but not necessarily causally linked, and may, in fact, be a measure of the disease process itself [4,7]. CRP has been detected within atherosclerotic plaques and causes endothelial dysfunction, oxidant stress, and intima hypertrophy in experimental models [8]. CRP is linked to subclinical atherosclerosis; intima media thickness (IMT), plaque presence, total plaque area (TPA) and vulnerable plaque characteristics in cross-sectional population studies. Our group has previously reported a cross-sectional relationship between CRP and TPA in men [9]. Only a few population-based studies have reported on the longitudinal association [10–13].

In the population based Tromsø study, we have repeatedly assessed CVD risk factors, serum CRP levels, plaque presence, and plaque characteristics in the carotid artery. In the present study, by novel utilization of linear mixed models and generalized estimating equations (GEE), we explore whether CRP has ability beyond traditional risk factors to predict novel plaque formation and plaque progression in men and women.

2. Materials and methods

2.1. Study population

The study participants were recruited from the 4th survey of the Tromsø study [14], a single-center, prospective, population-based health study of the inhabitants of Tromsø, Norway, carried out during the period 1994–1995. All inhabitants aged 55–74 years and 5–10% samples in other 5-year age groups (25–54 and 75–85 years) were offered an ultrasonographic examination of the right carotid artery and 6727 participants attended (76% of the eligible population). Participants not consenting to medical research (n = 40) and participants with limited ultrasound measurements (n = 3) were excluded. All participants still residing in Tromsø were invited to follow-up ultrasound examinations in the 5th (2001) and 6th (2007–2008) survey. Eligible for the present study were all subjects who participated in the carotid ultrasound examination in the 4th survey (1994–1995; baseline) and had CRP measurements and complete covariate information assessed at baseline (n = 6503). Of these, 4730 and 2917 were rescanned in the 5th and 6th survey, respectively, of whom 4590 participants from the 5th and 2838 participants from the 6th survey had valid measures on all covariates and outcomes and were included in the analyses. The maximal follow-up time was 13 years. Mean survey attendance was 2.2, and 2595 subjects had complete covariate and outcome information assessed at all three surveys. During follow-up (1994–2008), 1530 study participants died and 455 moved out of the municipality. Informed written consent was obtained from all participants. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Regional Committee for Medical and Health Research Ethics.

2.2. Carotid ultrasound examination

The baseline and follow-up measurements followed identical scanning and reading procedures. In 1994 and 2001, ultrasonography was performed with an Acuson Xp10 128 ART ultrasound scanner equipped with a 7.5 MHz linear-array transducer. In 2007, we used a GE Vivid 7 scanner with a linear 12-MHz transducer.

The far wall and near wall of the right common carotid artery, the bifurcation (bulb) and the internal carotid artery (six locations) were scanned for the presence of plaques. A plaque was defined as a localized thickening of the vessel wall of more than 50% compared with the adjacent intima-media thickness. Total plaque area (TPA) was calculated as the sum of all plaque areas. To ensure equal and standardized examination techniques and measurement procedures, sonographers completed a 2-month pre-study training protocol. Details about the inter- and intra-observer reproducibility and inter-equipment variability have been published previously [15–18].

2.3. Cardiovascular risk factors

Information on CVD risk factors was collected by physical examination, non-fasting blood samples and self-administered questionnaires. Blood pressure was recorded with an automatic device (Dinamap Vital Signs Monitor 1846; Critikon Inc. Tampa, FL, USA) by trained personnel. Participants rested for 2 min in a sitting position and then three readings were taken on the upper right arm at 1-min intervals. The average of the two last readings was used in the analyses. Non-fasting blood samples were collected from an ante-cubital vein. Serum was prepared by centrifugation after 1-h respite at room temperature and analyzed at the Department of Clinical Biochemistry, University Hospital of North Norway. Serum total cholesterol was analyzed by an enzymatic colorimetric method using a commercially available kit (CHOD-PAP, Boehringer-Mannheim, Mannheim, Germany). Serum high-density lipoprotein cholesterol (HDL-C) was measured after precipitation of lower-density lipoproteins with heparin and manganese chloride. Determination of glycosylated hemoglobin (HbA1c) in EDTA whole blood was based on an immunoturbidometric assay (UNIMATES, F. Hoffmann-La Roche AG). The HbA1c percent value was calculated from the HbA1c/Hb ratio. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). Information on former CVD (myocardial infarction and stroke), prevalent diabetes, current smoking and use of antihypertensive and lipid lowering drugs was collected from self-administered questionnaires. Diabetes was defined as self-reported diabetes, daily use of oral diabetic medication or insulin, or HbA1c levels >6.5%. CRP was analyzed in thawed aliquots after storage at − 70 °C (4th survey) or − 20 °C (5th and 6th surveys) with a particle-enhanced immunoturbidimetric assay on a Modular P (4th and 6th surveys) or Hitachi 917 (5th survey) autoanalyzer (Roche Hitachi, Mannheim, Germany), with reagents from Roche Diagnostics (Mannheim, Germany). Samples from the 4th survey were analyzed after 12 years of storage, and samples from the 5th and 6th surveys were analyzed in batches at the time of the surveys. The lower detection limit of the high-sensitivity CRP assay was 0.03 mg/L, and measurements of CRP lower than 0.03 mg/L were, therefore, set at this value. The analytical coefficient of variation for CRP levels between 0.1 mg/L and 20 mg/L was <4%. In the 6th survey, CRP was measured at 2 different time points and the average was used in the analyses.

Table 1
Sex-stratified, age-adjusted baseline characteristics by baseline CRP category. The Tromsø study 1994.

	Men (n = 3215)			Women (n = 3288)		
	CRP<1 mg/L	CRP 1–3 mg/L	CRP>3 mg/L	CRP<1 mg/L	CRP 1–3 mg/L	CRP>3 mg/L
Participants[a], %	38.5 (1237)	38.8 (1246)	22.8 (732)	44.9 (1475)	36.1 (1188)	19.0 (625)
Age[a], years	57.7 (57.1–58.2)	60.6 (60.1–61.2)	61.5[d] (60.8–62.2)	58.7 (58.2–59.2)	62.0 (61.4–62.5)	62.5[d] (61.7–63.3)
Systolic blood pressure, mmHg	143.4 (142.3–144.4)	144.9 (143.9–146.0)	147.4[d] (146.0–148.8)	141.8 (140.7–142.9)	146.1 (144.9–147.4)	150.9[d] (149.2–152.6)
Diastolic blood pressure, mmHg	84.0 (83.3–84.6)	84.9 (84.2–85.5)	86.3[d] (85.5–87.2)	80.3 (79.7–81.0)	82.6 (81.8–83.3)	83.2[d] (82.2–84.2)
Cholesterol, mmol/L	6.43 (6.36–6.50)	6.65 (6.59–6.72)	6.62[e] (6.54–6.71)	6.89 (6.83–6.95)	6.97 (6.90–7.04)	6.90 (6.81–7.00)
HDL-cholesterol, mmol/L	1.45 (1.43–1.47)	1.37 (1.35–1.39)	1.33[d] (1.30–1.36)	1.76 (1.74–1.78)	1.62 (1.60–1.65)	1.60[d] (1.56–1.63)
Body mass index, kg/m^2	25.4 (25.2–25.6)	26.5 (26.3–26.7)	26.5[d] (26.3–26.8)	24.7 (24.5–24.9)	26.7 (26.4–26.9)	27.7[d] (27.3–28.0)
Current smokers, %	24.3 (300)	37.5 (467)	47.3 (346)[d]	27.8 (410)	32.9 (391)	35.7 (223)[e]
Diabetes mellitus, %	3.1 (38)	3.4 (42)	6.8 (50)[d]	2.4 (35)	3.3 (39)	8.9 (56)[d]
Lipid-lowering medication, %	2.1 (26)	2.2 (27)	3.1 (23)	1.5 (22)	2.6 (31)	1.4 (9)
Antihypertensive medication, %	10.8 (134)	12.9 (161)	19.0 (139)[d]	10.4 (153)	14.8 (193)	18.9 (118)[d]
History of CVD, %	9.1 (113)	11.9 (148)	15.5 (112)[d]	4.1 (60)	5.3 (63)	8.0 (50)[d]
Carotid plaque, %	50.6 (626)	53.6 (668)	59.1 (433)[d]	42.8 (631)	45.8 (544)	50.4 (314)[e]
Total plaque area[b,c]	4.17 (4.02–4.32)	4.66 (4.52–4.79)	5.02[d] (4.84–5.18)	3.80 (3.68–3.92)	4.03 (3.90–4.15)	4.26[d] (4.05–4.38)

HDL, high density lipoprotein; CVD, cardiovascular disease.
The values are age-adjusted means (95% CI), or percentages (n).
[a] Unadjusted.
[b] In subjects with prevalent plaque.
[c] Square root transformed.
[d] *p*-value for linear trend across CRP risk categories <0.0001.
[e] *p*-value for linear trend across CRP risk categories <0.001.

2.4. Statistical analyses

We used the statistical software package SAS 9.4 (SAS Institute, Cary, NC) for all data analyses. Sex-stratified descriptive statistics are reported as means (standard deviations, SD), median (interquartile range, IQR) or percentages with numbers in brackets for each follow-up survey for all subjects (Supplemental Table 1). Differences in baseline characteristics between subjects who were lost to follow-up compared to subjects who completed follow-up examinations were assessed at each follow-up survey by *t*-test, Wilcoxon-Mann-Whitney or Chi-squared test (Supplemental Table 7). Age-adjusted sex differences in plaque presence and TPA at all surveys were assessed by logistic and linear regression. The associations between predefined CRP risk categories (CRP <1 mg/L, CRP 1–3 mg/L and CRP >3 mg/L) and CVD risk factors were examined in sex-stratified age-adjusted linear regression models (Table 1). Spearman correlation coefficient (r_s) was assessed for continuous variables. TPA was square root transformed (sqrtTPA) and CRP log transformed, to approximate normal distribution and improve regression model fit.

The cross-sectional association between CRP and presence of carotid plaque throughout the longitudinal study was assessed by generalized estimating equations (GEE) using a logit link function (Supplemental Table 2). Correlated observations within individuals were adjusted for by an exchangeable correlation structure [19]. The cross-sectional association between CRP and sqrtTPA throughout the longitudinal study was assessed in linear mixed models, adjusting for correlated observations within individuals by adding a random intercept to the model [19] (Supplemental Table 3). In both GEE and linear mixed model analyses, the associations were examined in separate models with CRP as a continuous variable and in risk categories (CRP <1, CRP 1–3 and CRP >3 mg/L) with CRP <1 mg/L as reference. In both GEE and linear mixed model analyses, the associations were examined in sex-stratified analyses firstly adjusting for age only, and then in a full model adjusting for CVD risk factors. CVD risk factors included were covariates reliably assessed in the Tromsø survey [14], which might act as confounders due to correlation with both CRP and TPA at baseline (age, smoking status, total cholesterol, HDL-C, systolic blood pressure, diabetes, BMI, and use of antihypertensive drugs) or a previously described association with both CRP and TPA (lipid-lowering drugs) [6,18].

In addition, a linear mixed model was used to simultaneously assess the cross-sectional and longitudinal relationship between CRP and sqrtTPA (Table 2) [20]. The cross-sectional component analyzed the association between baseline CRP and estimated sqrtTPA at baseline, whereas the longitudinal component analyzed the association between baseline CRP and sqrtTPA progression rate (CRP × time) during the observation period [21]. The models were fit with random intercepts and slopes. The association was first examined in sex-stratified analyses adjusting for baseline age, follow-up time and interaction terms (age × time and crp × time). Time was included as a continuous variable. In the fully adjusted models, CVD risk factors listed above and their corresponding interaction terms with time were additionally included. All continuous variables except TPA were grand mean centered and standardized before being included in the analyses. Dichotomous variables were included with absence of risk factor as reference. This facilitates interpretation of regression coefficients with regard to the intercepts (Table 2). The normality assumption was assessed by visual inspection of residuals.

The relationship between baseline CRP and future plaque

Table 2
Associations between baseline CRP and baseline TPA[a] and yearly TPA[a]-progression (slope), The Tromsø study 1994–2008.

	Men				Women			
	Subjects = 3215		Observations = 6821		Subjects = 3288		Observations = 7110	
	Age-adjusted		Multivariable-adjusted		Age-adjusted		Multivariable-adjusted	
	TPA[a]	Slope	TPA[a]	Slope	TPA[a]	Slope	TPA[a]	Slope
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Intercept	2.44 (2.36–2.53)	0.128 (0.118–0.138)	2.15 (2.03–2.27)	0.119 (0.106–0.133)	1.70 (1.63–1.77)	0.107 (0.098–0.115)	1.46 (1.36–1.56)	0.093 (0.081–0.105)
Baseline CRP[b]	0.28[d] (0.21–0.35)	0.005 (-0.003–0.013)	0.20[d] (0.12–0.27)	0.002 (-0.006–0.011)	0.11[d] (0.04–0.17)	0.002 (-0.005–0.008)	0.006 (-0.05–0.07)	−0.000 (-0.008–0.006)
Baseline CRP category[c]								
CRP < 1 mg/L	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
CRP 1–3 mg/L	0.36[d] (0.17–0.55)	0.010 (-0.010–0.032)	0.20[e] (0.01–0.39)	0.004 (-0.017–0.026)	0.20[e] (0.04–0.36)	−0.001 (-0.019–0.016)	0.02 (-0.13–0.17)	−0.008 (-0.026–0.009)
CRP >3 mg/L	0.86[d] (0.63–1.09)	0.029[e] (0.003–0.056)	0.55[d] (0.32–0.78)	0.018 (-0.009–0.045)	0.49[d] (0.29–0.68)	−0.017 (-0.025–0.022)	0.19 (-0.002–0.38)	−0.009 (-0.033–0.014)

TPA, total plaque area; β, regression coefficient; CI, confidence interval.
Age-adjusted: adjusted for baseline age and follow-up time. Multivariable-adjusted: adjusted for baseline age, total cholesterol, high density lipoprotein cholesterol, body mass index, diabetes, systolic blood pressure, smoking, lipid-lowering drugs, antihypertensive drugs and follow-up time. Intercept is for model with baseline CRP as continuous variable.
[a] Square root transformed.
[b] β and 95% CI for difference in baseline TPA and yearly change in TPA (slope) per 1 standard-deviation increase in baseline CRP. CRP was log transformed in analyses.
[c] β and 95% CI for difference in baseline TPA and yearly change in TPA (slope) for higher baseline CRP-risk categories compared to CRP <1 mg/L.
[d] *p*-value for β-coefficient <0.001.
[e] *p*-value for β-coefficient <0.05.

formation was assessed in subjects who were plaque-free at baseline in separate GEE analyses (Table 3). The covariates included were identical to covariates in the above models. Interaction terms with time were not included in the GEE models.

To address the impact of each CVD risk factor as a confounder in the relationship between CRP and subclinical atherosclerosis, we singly included each covariate in the age-adjusted models and evaluated the change in regression coefficients.

To ensure that our results were not confounded by former history of CVD and temporary inflammation, we repeated the analyses with exclusion of subjects with former CVD (N = 545) and observations of CRP>10 (N = 668). Analyses with TPA as outcome measure were repeated including only subjects with prevalent plaque at baseline. Analyses were rerun only in subjects who attended all three surveys (Supplemental Table 4-6).

3. Results

The baseline and follow-up characteristics of the study participants are listed in Supplemental Table 1. At all surveys, age-adjusted plaque prevalence and TPA were higher in men than in women ($p < 0.0001$). At baseline, 22.8% of men and 19.0% of women had CRP >3 mg/L. Table 1 displays age-adjusted CVD risk factors and carotid ultrasound findings across baseline CRP risk categories. Except for total cholesterol in women, there were positive linear trends of all CVD risk factors across CRP categories in both sexes. CRP and HDL-C were inversely correlated. The strongest correlations were between CRP and body mass index (BMI) (Spearman correlation coefficient (r_s) = 0.15 in men and 0.32 in women) and systolic blood pressure (r_s = 0.14 in men and 0.21 in women). CRP increased by age for both sexes (r_s = 0.17). Baseline plaque prevalence and TPA increased significantly across CRP risk categories in both sexes.

The cross-sectional association between CRP and plaque prevalence is shown in Supplemental Table 2. In age-adjusted models, there were significant associations between CRP and plaque prevalence for both sexes. Assessed on a continuous scale, multivariable-adjusted CRP was associated with plaque prevalence in men only. The association between CRP risk categories and plaque prevalence showed a less clear sex difference. For women, there was a significant increased plaque prevalence when CRP was >3 compared to CRP <1 mg/L (OR 1.20, CI 1.04–1.39). For men, this association was weaker (OR 1.15, CI 0.99–1.34). The cross-sectional association between CRP and TPA was significant in multivariable-adjusted analyses for both sexes, but strongest for men (Supplemental Table 3).

Table 2 shows the relationship between baseline CRP and baseline TPA as well as the effect of baseline CRP on TPA progression over time (slope). The intercepts represent baseline TPA and yearly increase in TPA for subjects with average CRP at baseline. Both baseline TPA and yearly increase in TPA were significantly higher in men than in women ($p < 0.002$). Baseline CRP was significantly associated with baseline TPA in both sexes ($p < 0.001$). In multivariable-adjusted analyses, these associations were evident in men only. For men, baseline CRP >3 mg/L was associated with increased TPA progression ($p = 0.03$) compared to baseline CRP <1 mg/L. However, in multivariable-adjusted models, baseline CRP did not predict TPA-progression in either sex. Age, systolic blood pressure and smoking remained significant predictors of TPA progression in both sexes in multivariable-adjusted analyses. In addition, use

Table 3
Associations between baseline CRP and novel plaque formation in subjects without plaque at baseline. The Tromsø study 1994–2008.

	Men		Women	
	Subjects = 1488	Observations = 3362	Subjects = 1798	Observations = 4148
	Age-adjusted	Multivariable-adjusted	Age-adjusted	Multivariable-adjusted
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Baseline CRP[a]	1.10 (1.01–1.21)[c]	1.05 (0.96–1.16)	1.03 (0.94–1.12)	0.99 (0.91–1.09)
Baseline CRP category[b]				
CRP <1 mg/L	Ref	Ref	Ref	Ref
CRP 1–3 mg/L	1.14 (0.91–1.44)	1.06 (0.83–1.34)	1.03 (0.83–1.29)	0.95 (0.76–1.18)
CRP >3 mg/L	1.44 (1.08–1.92)[c]	1.19 (0.88–1.58)	1.06 (0.80–1.41)	0.95 (0.71–1.28)

OR, odds ratio; CI, confidence interval.
Age-adjusted: adjusted for age and follow-up time. Multivariable-adjusted: adjusted for baseline age, total cholesterol, high density lipoprotein cholesterol, body mass index, diabetes, systolic blood pressure, smoking, lipid-lowering drugs, antihypertensive drugs and follow-up time.
[a] OR for novel plaque *vs.* no plaque at follow-up per 1 standard deviation increase in baseline CRP. CRP was log transformed in analysis.
[b] OR for novel plaque *vs.* no plaque at follow-up for higher baseline risk categories of CRP compared to CRP<1 mg/L.
[c] *p*-value for OR <0.05.

of lipid-lowering medication was a predictor in men and total cholesterol was a predictor in women only.

Among 3286 participants who had no plaque at baseline and attended a minimum of one follow-up study, 1304 (39.7%) participants formed at least one novel carotid plaque during follow-up. In men, who were plaque-free at baseline, the risk of novel plaque formation increased significantly by baseline level of CRP (Table 3). The risk for plaque at end of follow-up was 44% higher in men with baseline CRP >3 mg/L compared to men with baseline CRP <1 mg/L (OR 1.44, CI 1.08–1.92). However, this association was attenuated to non-significance upon adjustment for traditional risk factors. There was no association between baseline CRP and novel plaque formation in women. In multivariable-adjusted models, age, total cholesterol, systolic blood pressure, and smoking were predictors of novel plaque formation in both sexes. In addition, body mass index and use of lipid-lowering medication were predictors in men only.

Analyses assessing the impact of each CVD risk factor showed that no single risk factor changed the significance of the cross-sectional associations between CRP and atherosclerosis. HDL-C attenuated the cross-sectional association between CRP and TPA in women by 27%, but only 1.5% in men. Current smoking attenuated the regression coefficient by 16% in men and 11% in women. All other covariates had less influence on the regression coefficients. In longitudinal analyses, the predictive ability of baseline CRP for both TPA progression and novel plaque formation in men was attenuated to non-significance upon single adjustment for baseline smoking status. In addition, single adjustment for total cholesterol or systolic blood pressure had similar effects on CRPs association with novel plaque formation.

In multivariable-adjusted sensitivity analysis, where observations with CRP>10 mg/L and subjects with former CVD were excluded, results remained mainly unchanged with small variations in regression coefficients. When repeating the analyses with TPA as outcome measure for the subgroup with prevalent plaque at baseline, there was no change in the multivariable-adjusted results. The same was true when restricting the analyses to subjects who attended all three surveys (Supplemental Table 4–6).

4. Discussion

4.1. Cross-sectional associations

In cross-sectional analyses, we confirmed an association between CRP and carotid plaque prevalence as well as TPA. After adjustment for traditional CVD risk factors, these associations remained most prominent in men.

In small case-control trials, CRP has been linked to the presence of carotid artery stenosis [22,23]. The cross-sectional association between CRP and plaque presence is, however, not firmly established [9,11,24,25]. Disagreements may partly be explained by differences in methodological approaches, such as assessment-methods of carotid atherosclerosis, composition of study populations and degree of adjustment. Only a few CRP studies have reported on sex-stratified associations. Except for a cross-sectional study on the Framingham offspring [26], most of these studies support our findings and report a stronger association between subclinical carotid atherosclerosis and CRP in men [9,27–29].

Whether CRP reflects a response to traditional CVD risk factors or rises secondarily due to inflammatory processes within the atherosclerotic plaque is not clear. In our study and the above-mentioned studies, the associations between CRP and subclinical atherosclerosis were attenuated when controlling for CVD risk factors. CRP is associated with risk factors such as age, BMI, systolic blood pressure, cholesterol and smoking [30]. We found that smoking and HDL-C were the covariates with largest impact on the association between CRP and carotid atherosclerosis in men and women, respectively. Mechanisms relating smoking to CVD are not fully understood, but smoking-induced vascular endothelial dysfunction, inflammation and development of atherosclerosis is suspected to play an important role. HDL-C is inversely correlated with subclinical atherosclerosis, clinical CVD and CRP and considered to be a stronger risk factor for CVD in women than in men. Proposed protective mechanisms are inflammatory modulating effects and reverse cholesterol transport by HDL-C [31]. Ben-Yehuda claims that although vascular inflammation may contribute to an elevation of CRP in the blood, CRP is mainly linked to abdominal obesity and insulin resistance [32]. Abdominal adipocytes produce inflammatory cytokines including interleukin-6, which is a potent messenger for CRP secretion in the liver [32]. In our study, CRP was correlated with BMI, but the cross-sectional relationship between CRP and subclinical atherosclerosis was minimally attenuated by BMI. However, results from the Multi-Ethnic Study of Atherosclerosis indicate that in the absence of obesity, CRP is not associated with coronary calcium and only weakly associated with IMT, whereas obesity was related to both imaging outcomes [33], suggesting a complex interplay between metabolic disorders, inflammation and serum lipids in atheroma formation.

CRP is the marker of inflammation most extensively studied in relation to CVD, and is usually selected due to its analytical advantages and stability in regard to short-term fluctuations [34]. The

long-term stability of CRP values (within-person correlation coefficient, 0.59; 95% CI, 0.52 to 0.66) is comparable to that of both blood pressure and total serum cholesterol [5]. However, CRP is a downstream marker of inflammation, which rises in most situations of acute infection and inflammation [22,23]. Plaque inflammation assessed by FDG-PET [35,36] or immune pathological analysis [37] was not found to be associated with CRP. In addition, results regarding CRPs associations with unstable plaque features such as echogenicity have been contradictory [9,22]. Although CRP is associated with prevalent atherosclerosis beyond traditional risk factors, other circulating inflammatory markers may better reflect the inflammatory process within the plaque, and thereby show higher sensitivity and specificity for the detection and monitoring of inflammatory atherosclerotic disease [1,38,39].

4.2. Longitudinal associations

In age-adjusted models, baseline CRP predicted TPA progression and novel plaque formation in men, but not in women. However, the predictive ability disappeared after adjustment for conventional risk factors. These results suggest that information added by CRP in prediction of progressive atherosclerotic disease addressed to the presence of other risk factors, such as systolic blood pressure, smoking and total cholesterol. In other prospective studies, baseline CRP predicted progressive atherosclerotic disease defined as increase in plaque score and progression of stenosis [10,40,41]. In a study of older subjects, CRP was an independent predictor of new carotid plaques within three years [12]. The Austrian Stroke Prevention Study demonstrated a significant relationship between baseline CRP and baseline carotid atherosclerosis, as well as progression of atherosclerosis during the observational period of 6 years [11]. However, CRP did not predict progression of IMT in a meta-analysis compromising 20 studies and 49 097 subjects [13].

We found no evidence of elevated CRP levels proceeding novel plaque formation or plaque progression in women. Anti-inflammatory effects of female sex-hormones may shift the atherosclerotic process in women toward a less inflammatory and slower progressive development [42]. Lower prevalence and progression rate in women may have reduced statistical power to detect an association between CRP and progressive atherosclerosis.

A large body of evidence documents an independent relationship between CRP and increased risk of CVD events. Whether CRP is merely a risk marker or a causal factor of atherosclerosis and ischemic vascular disease remains to be clarified [43]. The clinical utility of measuring risk factors resides in the fact that treatment may directly modify risk [44]. Although causal interferences cannot be drawn from this epidemiologic study, our results do not support an independent role of CRP in the formation and growth of atherosclerotic plaques. These findings are consistent with a recent review article by Ridker [39]. Mendelian randomization studies both in the Copenhagen study [45], and in a combined study of 194,418 participants, including 46, 557 patients with prevalent or incident coronary heart disease [46], concluded that CRP gene variants associated with increased CRP levels did not lead to increased risk of ischemic atherosclerotic disease. In addition, several mouse studies did not find evidence of a causal role of CRP in atherosclerotic development [47,48]. If elevated CRP does not proceed plaque progression or formation, it is unlikely that reducing CRP-levels will affect progression of subclinical atherosclerosis and CRPs role as a therapeutic target in this regard may be limited. In accordance with these findings, the proposed usefulness of CRP measurements in predicting benefit from statin treatment [6] has later been drawn in doubt by results from the ASCOT [49] and Heart Protection Study [50].

4.3. Strengths and limitations

To our knowledge, this is the first longitudinal study using linear mixed models to utilize information from repeated measures on CRP and carotid plaque. The mixed model allows inclusion of observations from subjects who do not have complete follow-up data and thereby reduces loss to follow-up bias. The model utilizes information from time-changing exposure variables and diminishes the regression dilution effect. It also takes into account the dependency of observations made on the same individual over time. Bias related to the inclusion of baseline measurements in the change-models is addressed by assessing the estimated baseline in the models [21]. Mixed models are well suited to address different aspects of how risk factors influence the development of subclinical atherosclerosis over time when repeated measures of risk factors and outcome are available. Strengths of the present study are the high attendance rates, large sample size and high validity of outcome measurements. CRP has a poor specificity in the presence of a coexisting inflammatory condition such as rheumatoid arthritis and infections. In sensitivity analysis, we aimed at minimizing this source of error by excluding observations with CRP>10 mg/L and subjects with prevalent CVD at baseline.

Limitations of the study are related to loss of follow-up, which may have attenuated the results towards null, as subjects with the most unfavorable baseline risk factor and atherosclerosis levels were more likely to drop out from follow-up examinations (Supplemental Table 7). We expect the relationship between atherosclerosis and CRP to be equivalent in attendants and non-attendants. However, loss to follow-up of subjects with most pronounced atherosclerosis progression might have reduced statistical power to detect an association. Although we used a standardized protocol for the measurement of TPA, a relatively large part of the computed change in TPA over the years may be distorted by measurement error. The use of different ultrasonography equipment in the 4th and the 6th survey, and non-standardized uptake angles is likely to have increased the measurement error. Regression to the mean may have affected our outcome measure and plaques of low echogenicity may have been overlooked. Any such misclassification would be expected to underestimate the true association. Residual confounding may exist, and it cannot be ruled out that shorter intervals between surveys could have led to different results regarding the predictive value of CRP.

4.4. Conclusion

In conclusion, we found a cross-sectional association between CRP and prevalent plaque as well as TPA that was stronger in men than in women and independent of traditional CVD risk factors. Baseline CRP was not a predictor of novel plaque formation nor TPA progression in either men or women, when adjusted for traditional risk factors. Our results cast doubt that CRP plays a causal role in the initiation and progression of atherosclerosis, and suggest that CRP may be linked to CVD by other mechanisms. Our findings indicate that CRP may be considered as a potential tool to identify subjects with prevalent atherosclerosis, but question its role as a therapeutic target in haltering progressive atherosclerotic disease.

Conflict of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

Financial support

The Tromsø study has been supported by the Research Council

of Norway, the Norwegian Council on Cardiovascular Disease, the Northern Norway Regional Health Authority; the University of Tromsø, the Norwegian Foundation for Health and Rehabilitation, the Odd Berg Research Foundation and the Simon Fougner Hartmann's Family Fund. Agnethe Eltoft receives a research grant from the University Hospital of North Norway,Tromsø, Norway.

Author contributions

A. Eltoft analyzed and interpreted the data and drafted the manuscript. S.H. Johnsen and E.B. Mathiesen conceived, designed and supervised the research. J.B. Hansen and E.B. Mathiesen handled funding. K.A. Arntzen, J.B. Hansen, S.H. Johnsen and E.B. Mathiesen acquired the data. T. Wilsgaard contributed to data analysis. K.A. Arntzen, J.B. Hansen, E.B. Mathiesen, T. Wilsgaard and S.H. Johnsen made critical revision of the manuscript.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atherosclerosis.2017.07.001.

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Supplemental Table 1. Crude characteristics of the study participants across surveys stratified by sex. The Tromsø Study 1994-2008.

	Tromsø 4 (n=6503)		Tromsø 5 (n=4590)		Tromsø 6 (n=2828)	
	Men n= 3215	Women n= 3288	Men n= 2234	Women n= 2356	Men n= 1372	Women n= 1466
Age, years	59.7 (10.0)	60.6 (10.3)	65.9 (9.3)	67.0 (9.8)	69.0 (9.0)	69.7 (10.0)
Systolic blood pressure, mmHg	144.9 (20.4)	145.1 (24.5)	143.2 (20.5)	144.8 (23.0)	145.1 (21.2)	148.2 (26.4)
Diastolic blood pressure, mmHg	84.9 (12.2)	81.7 (13.5)	82.45 (11.8)	80.7 (13.2)	80.5 (10.2)	76.2 (10.6)
Cholesterol, mmol/L	6.56 (1.20)	6.92 (1.34)	6.07 (1.14)	6.51 (1.18)	5.45 (1.13)	5.91 (1.11)
HDL-cholesterol, mmol/L	1.39 (0.39)	1.68 (0.43)	1.35 (0.37)	1.60 (0.40)	1.40 (0.41)	1.70 (0.44)
CRP [a], mg/L	1.32 (0.69-2.76)	1.15 (0.57-2.44)	1.64 (0.87-3.20)	1.53 (0.79-3.10)	1.61 (0.93-2.98)	1.57 (0.83-3.03)
Body mass index, kg/m^2	26.1 (3.4)	26.0 (4.5)	26.8 (3.5)	26.8 (4.6)	27.2 (3.5)	26.8 (4.5)
Current smokers, %	34.7 (1114)	31.1 (1024)	25.3 (565)	23.8 (560)	14.6 (200)	15.6 (228)
Diabetes mellitus, %	4.1 (131)	4.0 (130)	10.2 (227)	8.1 (190)	10.8 (148)	10.1 (148)
Lipid-lowering medication, %	2.4 (76)	1.9 (61)	17.3 (387)	12.8 (302)	29.5 (405)	22.0 (323)
Antihypertensive medication, %	13.5 (433)	13.6 (447)	25.3 (566)	26.1 (615)	33.1 (454)	37.2 (546)
History of CVD %	11.6 (372)	5.3 (173)	16.3 (363)	8.1 (190)	20.3 (279)	9.6 (140)
Carotid plaque present, %	53.7 (1727)	45.3 (1490)	67.5 (1507)	57.1 (1344)	61.0 (837)	52.2 (765)
Total plaque area [ab], mm^2	17.6 (10.0-31.7)	13.6 (7.0-23.3)	20.8 (11.6-37.0)	16.6 (9.3-27.6)	26.6 (14.8-43.6)	20.4 (12.0-31.7)

HDL; high density lipoprotein. CVD; cardiovascular disease. The values are unadjusted means (standard deviations), or percentages (n).
[a] Median (interquartile range) due to skewed distribution. [b] In subjects with prevalent plaque.

Supplemental Table 2: Cross-sectional associations between CRP and plaque prevalence. The Tromsø Study 1994-2008.

	Men Subjects = 3215 Observations = 6821		Women Subjects = 3288 Observations = 7110	
	Age-adjusted OR (95 % CI)	Multivariable-adjusted OR (95 % CI)	Age-adjusted OR (95 % CI)	Multivariable-adjusted OR (95 % CI)
CRP [a]	1.10 (1.05-1.16) [c]	1.08 (1.02-1.13) [d]	1.07 (1.02-0.12) [d]	1.04 (0.99-1.10)
CRP category [b]				
CRP <1 mg/L	Ref	Ref	Ref	Ref
CRP 1-3 mg/L	1.14 (1.02-1.27) [d]	1.09 (0.97-1.23)	1.14 (1.02-1.28) [d]	1.10 (0.97-1.23)
CRP >3 mg/L	1.25 (1.08-1.44) [d]	1.15 (0.99-1.34)	1.26 (1.10-1.44) [c]	1.20 (1.04-1.39) [d]

OR; odds ratio. CI; Confidence interval.
[a] OR are for novel plaque versus no plaque per 1 standard deviation increase in CRP. CRP was log transformed in analyses.
[b] OR are for novel plaque versus no plaque for higher risk categories of CRP compared to CRP <1 mg/L.
Multivariable adjusted: adjusted for age, total cholesterol, high density lipoprotein cholesterol, body mass index, diabetes, systolic blood pressure, smoking, lipid-lowering drugs and antihypertensive drugs. P- value [c] <0.001, [d] <0.05.

Supplemental Table 3: Cross-sectional associations between CRP and total plaque area. The Tromsø Study 1994-2008.

	Men Subjects = 3215 Observations = 6821		Women Subjects = 3288 Observations = 7110	
	Age-adjusted β (95 % CI)	Multivariable-adjusted β (95 % CI)	Age-adjusted β (95 % CI)	Multivariable-adjusted β (95 % CI)
CRP [a]	0.17 (0.12-0.23) [c]	0.16 (0.10-0.21) [c]	0.10 (0.05-0.14) [c]	0.08 (0.03-0.12) [d]
CRP category [b]				
CRP <1 mg/L	Ref	Ref	Ref	Ref
CRP 1-3 mg/L	0.21 (0.09-0.34) [c]	0.18 (0.05-0.31) [d]	0.18 (0.08-0.29) [c]	0.14 (0.03-0.25) [d]
CRP >3 mg/L	0.47 (0.32-0.62) [c]	0.40 (0.24-0.55) [c]	0.32 (0.19-0.45) [c]	0.26 (0.13-0.40) [c]

β; regression coefficient. CI; Confidence interval. Total plaque area was square root transformed.
[a] β -coefficients for difference in sqrtTPA (95 % CI) per 1 standard deviation increase in CRP. CRP was log transformed in analyses.
[b] β -coefficients for difference in sqrtTPA (95 % CI) for higher risk categories of CRP compared to CRP <1 mg/L.
Multivariable-adjusted: adjusted for age, total cholesterol, high density lipoprotein cholesterol, body mass index, diabetes, systolic blood pressure, smoking, lipid-lowering drugs and antihypertensive drugs. P-value for β –coefficient [c] <0.001, [d] <0.05.

Supplemental table 4. Crude characteristics of the study participants across surveys in men (n=1265) and women (n=1330) who attended all surveys. The Tromsø Study 1994-2008.

	Tromsø 4		Tromsø 5		Tromsø 6	
	Men	Women	Men	Women	Men	Women
Age, years	56.3 (8.7)	57.1 (9.7)	63.3 (8.7)	64.1 (9.7)	69.3 (8.7)	70.1 (9.7)
Systolic blood pressure, mmHg	140.2 (18.1)	138.9 (21.6)	140.3 (19.4)	141.2 (22.1)	145.3 (21.1)	148.7 (26.4)
Diastolic blood pressure, mmHg	83.6 (11.4)	79.6 (12.3)	82.0 (11.3)	79.7 (12.4)	80.5 (10.2)	76.3 (10.7)
Cholesterol, mmol/L	6.54 (1.15)	6.72 (1.29)	6.14 (1.13)	6.44 (1.16)	5.45 (1.12)	5.92 (1.11)
HDL-cholesterol, mmol/L	1.38 (0.38)	1.70 (0.40)	1.35 (0.36)	1.60 (0.40)	1.40 (0.42)	1.70 (0.44)
CRP [a], mg/L	1.07 (0.58-2.12)	0.97 (0.48-1.92)	1.48 (0.82-2.83)	1.46 (0.76-3.06)	1.59 (0.92-2.91)	1.58 (0.83-3.06)
Body mass index, kg/m^2	26.1 (3.0)	25.6 (3.9)	27.0 (3.3)	26.7 (4.2)	27.2 (3.5)	26.8 (4.5)
Current smokers, %	28.0 (354)	27.1 (360)	22.3 (282)	21.7 (289)	14.2 (179)	15.2 (202)
Diabetes mellitus, %	1.6 (20)	2.1 (28)	6.7 (85)	6.7 (89)	10.8 (137)	10.0 (133)
Lipid-lowering medication, %	2.1 (27)	1.3 (17)	16.2 (205)	11.7 (155)	29.3 (370)	22.0 (293)
Antihypertensive medication, %	7.4 (94)	2.6 (34)	19.5 (247)	22.3 (296)	33.1 (419)	37.7 (501)
History of CVD %	6.3 (79)	8.4 (112)	12.1 (153)	5.34 (71)	20.7 (262)	9.4 (125)
Carotid plaque present, %	43.2 (546)	34.2(455)	60.1 (760)	48.0 (638)	61.2 (774)	52.1 (693)
Total plaque area [ab], mm^2	14.1 (8.5-24.7)	11.4 (6.7-19.6)	17.9 (10.6-32.2)	15.3 (8.6-24.4)	26.7 (14.7-44.0)	20.2 (12.0-31.7)

HDL; high density lipoprotein. CVD; cardiovascular disease. The values are unadjusted means (standard deviations), or percentages (n).[a] Median (interquartile range) due to skewed distribution. [b] In subjects with prevalent plaque.

Supplemental Table 5: Associations between baseline CRP and novel plaque formation in subjects without plaque at baseline who attended all surveys. The Tromsø Study 1994-2008.

	Men Subjects = 719 Observations = 2157		Women Subjects = 875 Observations = 2625	
	Age-adjusted OR (95 % CI)	Multivariable-adjusted OR (95 % CI)	Age-adjusted OR (95 % CI)	Multivariable-adjusted OR (95 % CI)
Baseline CRP [a]	1.10 (0.99-1.21)	1.04 (0.93-1.16)	0.99 (0.90-1.12)	0.96 (0.87-1.06)
Baseline CRP category [b]				
CRP <1 mg/L	Ref	Ref	Ref	Ref
CRP 1-3 mg/L	1.07 (0.82-1.41)	0.99 (0.75-1.30)	1.03 (0.78-1.35)	0.90 (0.68-1.20)
CRP >3 mg/L	1.44 (0.98-2.09)	1.20 (0.82-1.76)	0.98 (0.68-1.44)	0.86 (0.58-1.29)

OR; odds ratio. CI; confidence interval.

[a] OR for novel plaque versus no plaque at follow-up per 1 standard deviation increase in baseline CRP. CRP was log transformed in analysis.
[b] OR for novel plaque versus no plaque at follow-up for higher baseline risk categories of CRP compared to CRP<1 mg/L.
Age-adjusted: adjusted for age and follow-up time. Multivariable adjusted: adjusted for baseline age, total cholesterol, high density lipoprotein cholesterol, body mass index, diabetes, systolic blood pressure, smoking, lipid-lowering drugs, antihypertensive drugs and follow-up time.

Supplemental Table 6: Associations between baseline CRP and baseline TPA[a] and TPA[a]-progression (slope) over time in men and women who attended all surveys. The Tromsø Study 1994-2008.

	Men Subjects = 1265 Observations = 3795				Women Subjects = 1330 Observations = 3990			
	Age-adjusted		Multivariable-adjusted		Age-adjusted		Multivariable-adjusted	
	TPA[a] β (95 % CI)	Slope β (95 % CI)	TPA[a] β (95 % CI)	Slope β (95 % CI)	TPA[a] β (95 % CI)	Slope β (95 % CI)	TPA[a] β (95 % CI)	Slope β (95 % CI)
Intercept	2.06 (1.93-2.18)	0.13 (0.12-0.14)	1.78 (1.61-1.95)	0.11 (0.09-0.13)	1.40 (1.30-1.51)	0.10 (0.90-0.11)	1.21 (1.07-1.36)	0.08 (0.065-0.093)
Baseline CRP [b]	0.14 [d] (0.05-0.23)	0.006 (-0.003-0.014)	0.07 (-0.02-0.16)	0.003 (-0.006-0.011)	0.02 (-0.05-0.10)	0.002 (-0.053-0.103)	-0.049 (-0.13-0.28)	0.0006 (-0.007-0.008)
Baseline CRP category [c]								
CRP < 1 mg/L	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
CRP 1-3 mg/L	0.14 (-0.12-0.39)	0.013 (-0.019-0.030)	-0.02 (-0.28-0.23)	-0.0003 (-0.025-0.024)	0.21 (-0.01-0.42)	0.000 (-0.02-0.02)	0.02 (-0.20-0.23)	-0.005 (-0.026-0.015)
CRP >3 mg/L	0.68 [d] (0.34-1.02)	0.026 (-0.007-0.059)	0.39 [d] (0.05-0.73)	0.012 (-0.021-0.046)	0.25 (-0.05-0.51)	0.000 (-0.03-0.03)	0.02 (-0.27-0.32)	-0.003 (-0.033-0.026)

TPA; Total plaque area. β; regression coefficient. CI; confidence interval.[*]Square root transformed.

[b] β and 95 % CI for difference in baseline TPA and yearly change in TPA (slope) per 1 standard-deviation increase in baseline CRP. CRP was log transformed in analyses.

[c] β and 95 % CI for difference in baseline TPA and yearly change in TPA (slope) for higher baseline CRP-risk categories compared to CRP <1 mg/l.

Age-adjusted: adjusted for baseline age and follow-up time. Multivariable-adjusted: adjusted for baseline age, total cholesterol, high density lipoprotein cholesterol, body mass index, diabetes, systolic blood pressure, smoking, lipid-lowering drugs, antihypertensive drugs and follow-up time. Intercept for model with baseline CRP as continuous variable.
[d] P-value for β-coefficient <0.05

Supplemental Table 7. Differences in baseline characteristics between attendants and non-attendants by follow-up surveys. The Tromsø Study 1994-2008.

	Tromsø 5			Tromsø 6		
	Attendants (n=4730)	Non-attendants (n=1773)	p-value	Attendants (n=2917)	Non-attendants (n=3586)	p-value
Age, years	59.5 (9.6)	62.1 (11.4)	<0.0001	56.4 (9.5)	63.2 (9.7)	<0.0001
Men, % [c]	48.4 (2290)	52.2 (925)	0.007	48.4 (1212)	50.3 (1803)	ns
Systolic blood pressure, mmHg	143.6 (21.6)	148.7 (24.5)	<0.0001	139.6 (20.1)	149.4 (23.4)	<0.0001
Diastolic blood pressure, mmHg	83.0 (12.5)	84.0 (14.0)	0.005	81.4 (12.0)	84.7 (12.0)	<0.0001
Cholesterol, mmol/L	6.74 (1.26)	6.76 (1.36)	ns	6.63 (1.24)	6.84 (1.32)	<0.0001
HDL-cholesterol, mmol/L	1.55 (0.43)	1.52 (0.44)	0.017	1.53 (0.44)	1.55 (0.42)	ns
CRP [a], mg/L	1.11 (0.57-2.24)	1.68 (0.81-3.58)	<0.0001[d]	1.04 (0.54-2.02)	1.44 (0.71-3.08)	<0.0001
Body mass index, kg/m^2	26.0 (3.7)	26.0 (4.5)	ns	25.9 (3.5)	26.1 (4.3)	0.013
Current smokers, % [c]	30.2 (1429)	40.0 (709)	<0.0001	28.0 (816)	36.9 (1322)	<0.0001
Diabetes mellitus, % [c]	3.0 (140)	6.8 (121)	<0.0001	3.0 (140)	6.8 (121)	<0.0001
Lipid-lowering medication, % [c]	2.3 (17)	2.1 (27)	ns	1.7 (50)	2.4 (87)	0.047
Antihypertensive medication, %	6.3 (299)	18.1 (321)	<0.0001	8.7 (255)	17.4 (625)	<0.0001
History of CVD %	8.4 (545)	13.9 (246)	<0.0001	4.4 (127)	11.7 (418)	<0.0001
Carotid plaque present, % [c]	33.5 (2180)	58.5 (1037)	<0.0001	38.6 (1125)	58.3 (2092)	<0.0001
Total plaque area [ab], mm^2	14.3 (8.4-24.8)	19.2 (10.3-32.9)	<0.0001[d]	12.6 (7.5-21.6)	17.4 (10.0-31.2)	<0.0001

HDL; high density lipoprotein. CVD; cardiovascular disease. Values are unadjusted means (standard deviations), or percentages (n). [a] Median (interquartile range) due to skewed distribution. [b] In subjects with prevalent plaque. p-value is for equality between groups tested by t-test,[c] chi square test. [d] Wilcoxon-Mann-Whitney. Ns; non-significant with p-value > 0.05.