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Clinical correlates of change in inflammatory biomarkers: The Framingham Heart Study

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Abstract

Objectives—Traditional clinical risk factors are associated with inflammation cross-sectionally, but associations of longitudinal variation in inflammatory biomarkers with corresponding changes in clinical risk factors are incompletely described. We sought to analyze clinical factors associated with change in inflammation in the community.

Methods—We studied 3013 Framingham Offspring (n = 2735) and Omni Cohort (n = 278) participants (mean age 59 years, 55% women, 9% ethnic/racial minority) who attended two consecutive examination cycles (mean 6.7 years apart). We selected ten inflammatory biomarkers representing distinctive biological functions: C-reactive protein (CRP), intercellular adhesion molecule-1, interleukin-6, isoprostanes, lipoprotein-associated phospholipase-2 (Lp-PLA2) activity, Lp-PLA2-mass, monocyte chemoattractant protein-1, osteoprotegerin, P-selectin, and tumor necrosis factor receptor II (TNFRII). We constructed multivariable-adjusted regression models to assess the relations of baseline, follow-up and change in clinical risk factors with change in biomarker concentrations over time.

Results—Baseline, follow-up and change in clinical risk factors explain a moderate amount of the variation in biomarker concentrations across 2 consecutive examinations (ranging from r^2 = 0.28 [TNFRII] up to 0.52 [Lp-PLA2-mass]). In multivariable models, increasing body-mass index, smoking initiation, worsening lipid profile, and increasing waist size were associated with increasing concentrations of several biomarkers. Conversely, hypercholesterolemia therapy and

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Conflict of interest

Lp-PLA2 activity measurements were provided by GlaxoSmithKline and mass measurements by diaDexus at no cost to the FHS.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atherosclerosis.2013.01.019.

hormone replacement cessation were associated with decreasing concentrations of biomarkers such as CRP, Lp-PLA2-mass and activity.

Conclusion—Cardiovascular risk factors have different patterns of association with longitudinal change in inflammatory biomarkers and explain modest amounts of variability in biomarker concentrations. Nevertheless, a substantial proportion of longitudinal change in inflammatory markers is not explained by traditional risk factors.

Keywords

Biological markers; Longitudinal studies; Inflammation

1. Introduction

Inflammation and CVD risk factors are closely interrelated. CVD risk factors, such as hypertension, and hypercholesterolemia are associated with higher circulating concentrations of inflammatory biomarkers. Conversely, chronic inflammation increasingly is recognized as a major contributor to the development and the progression of atherosclerosis, CVD risk factors [1, 2] and subsequent cardiovascular disease (CVD) [3, 4]. Prior studies have reported the relations of CVD risk factors to circulating inflammatory biomarkers assessed on a single occasion [5–7].Nevertheless, there are only a few observational studies available examining the longitudinal progression of circulating inflammatory biomarkers [8]. Understanding modulators of longitudinal progression in inflammation potentially may improve early recognition of atherosclerosis and enhance risk prediction of CVD beyond traditional clinical risk factors [4].

Recent studies reveal an alarming increasing prevalence of CVD risk factors globally [9–11]. As risk factors and inflammation are closely associated, both may contribute to increased CVD burden. We sought to investigate the risk factors related to the progression of 10 inflammatory biomarkers representing distinctive biological functions. We hypothesized that higher level of CVD risk factors is associated with greater systemic inflammation and, therefore, increased biomarker levels. Further, we postulated that adverse changes in clinical factors would be associated with accelerated inflammation. We tested these hypotheses in a large community-based sample.

2. Methods

2.1. Study sample

The study design and selection criteria of the Framingham Offspring Study and Framingham Omni Study have been described previously [12, 13]. Since enrollment in 1971 (Offspring) and 1994 (Omni), study participants have had regular follow-up every 4–8 years. Offspring Cohort participants who attended the seventh (n = 3,539, 1998–2001, of which n = 3334 had a clinical examination) and eighth examination cycles (n = 3021, 2004–2008, of which n = 2913 had a clinical examination), or Omni participants attending the second (n = 405, 1999–2001) and third (n = 298, 2007–2008), were eligible for the study if they had at least one biomarker measured at both examination cycles (separated by 3.7–8.7 years). We excluded participants for the following reasons: missing biomarker measurement (n = 136) and missing covariate data (n = 246). The Boston University Medical Center Institutional Review Board approved the study protocol. All participants provided informed consent at both examinations.

2.2. Clinical evaluations

The Framingham Heart Study clinical evaluations were standardized and consisted of a concise clinical history with emphasis on physician ascertainment of symptoms, lifestyle habits, medications, interim cardiovascular events and a focused cardiovascular physical examination. Research technicians measured height, weight and waist circumference. Body-mass index was calculated as weight measured in kilograms divided by the square of height in meters (kg/m^2). Current smoking was defined as self-report of smoking cigarettes regularly in the year prior to examination. Moderate to heavy alcohol consumption was defined as having reported ≥14 drinks per week for men, and ≥7 drinks per week for women. Blood samples were drawn after an overnight fast.

2.3. Biomarkers measurements

Participants were fasting except for the Omni Cohort at their examination cycle two [14]. We processed, centrifuged and stored blood specimens at −80 °C until assays were performed. We selected 10 different biomarkers of vascular inflammation available in the Framingham Heart Study: C-reactive protein (CRP), soluble intercellular adhesion molecule-1 (sICAM-1), interleukin-6, urinary 8-epi-PGF2α isoprostanes (isoprostanes), lipoprotein-associated phospholipase-A2 (Lp-PLA2) activity, Lp-PLA2-mass, monocyte chemoattractant protein-1 (MCP-1), osteoprotegerin, P-selectin, and tumor necrosis receptor II (TNFRII). CRP was measured using particle enhanced immunonephelometry; sICAM-1, interleukin-6, isoprostanes, Lp-PLA2-mass and activity, MCP-1, P-Selectin, and TNFRII were assayed with ELISA. We performed standard quality control evaluations; all intra-assay coefficients of variation were <10%. The details of the assays and measurement of were described elsewhere [14–16] and the biomarkers measurement manuals can be found at the FHS website http://www.framinghamheartstudy.org/share/vascularprotocols.html.

2.4. Statistical analysis

All analyses were conducted using natural logarithmically-transformed biomarkers to normalize their skewed distributions. To facilitate interpretation of concentrations of biomarkers between the two consecutive examinations we calculated medians and quartiles of each biomarker on the original scale. Pearson correlations were estimated between biomarker concentrations from the two consecutive examinations. For each biomarker we defined the response variable as change in biomarker concentration between examinations. We fitted multivariable regression models of change versus baseline biomarker level as well as traditional clinical factors – baseline and changes between examinations for each biomarker. We standardized both the change in markers and continuous clinical variables to mean 0 and SD 1 to facilitate the comparison of estimated beta coefficients across biomarkers. We examined stepwise models with grouped variables for most covariates: for continuous measures we used baseline value and change in value; for binary traits we used baseline status and change in status. After selecting groups of variables for each biomarker, we ran backward elimination to remove some nonsignificant individual covariates from groups. We selected covariates typically available in the clinical setting and known to be associated with CVD. Candidate variables were smoking, alcohol use, systolic blood pressure, diastolic blood pressure, body-mass index, waist circumference, glucose level, total/high-density lipoprotein (HDL) cholesterol level, low-density lipoprotein (LDL) level, use of aspirin, hormone replacement therapy, treatment for hypertension, treatment for hypercholesterolemia, history of diabetes, and history of cardiovascular disease. To facilitate comparisons among markers, we standardized response and (continuous) predictor variables. Given multiple tests, we used a 50-fold Bonferroni correction and considered a two-sided $p \leq 0.001$ as statistically significant in each analysis. With sample size 3000 and significance level 0.001, we had power 0.80 to detect association between clinical variables with change in ln(biomarker) if the true partial correlation was 0.076 or larger in absolute value; for

isoprostanes, sample size was $n = 2480$ and the corresponding partial correlation was 0.083. All analyses were performed using SAS 9.2 (SAS Institute, Cary, North Carolina).

3. Results

We examined 3013 participants (55% women, mean age 59 ± 9 years, 9% ethnic/racial minorities) from the Framingham Heart Study Offspring and Omni Cohorts (Table 1). Among participants who attended the index examination and who had biomarker and covariate data, 274 died before the close of examination 8 (mean age 69 years, 71% had hypertension, 30% had diabetes, 18% had CVD). Over the mean 6.7 year interval between baseline and follow-up examinations the prevalence of medication use (antihypertensive, lipid-lowering medication and aspirin) increased in both men and women. The only exception observed was hormone replacement therapy in women, which decreased. Fig. 1 illustrates the distribution of change of biomarkers on follow-up examination. Supplementary eFigure 1 compares the Box plots for change in log-transformed biomarkers after removing outliers. The median concentrations at baseline and at follow-up of each biomarker are shown in Table 2. The baseline and follow-up concentration of each biomarker were correlated; the lowest correlation was 0.32 for isoprostanes and the highest correlation was 0.66 for osteoprotegerin (eTable 1). By and large, the correlation between change in ln concentration of biomarkers was weak, except for interleukin-6 and CRP ($r = 0.43$) and Lp-PLA2 activity and mass ($r = 0.55$) (Table 3).

3.1. Clinical correlates of change of biomarkers

The results of the multivariable models of longitudinal change in biomarkers are shown in Table 4. Overall, we observed that the most consistent and strongest factor associated with change in biomarker levels was the baseline biomarker concentration ($\beta = -0.736$; SE = 0.015 for isoprostanes to $\beta = -0.193$; SE = 0.010 for TNFRII). Higher baseline biomarker concentration was associated with lower biomarker concentration at follow-up.

We accounted for demographic factors (age, sex, cohort status) in all models. For most biomarkers, the changes in biomarker concentrations were similar for men and women. However, sICAM-1 and isoprostanes serially increased more in women compared to men ($\beta = 0.127$, SE = 0.132; $\beta = 0.234$, SE = 0.028, respectively). Older age at the baseline examination was associated with increasing concentrations of biomarkers for six of the biomarkers, including CRP, interleukin-6, sICAM-1, MCP-1, osteoprotegerin and TNFRII, with osteoprotegerin increasing the most with older participant age ($\beta = 0.278$, SE = 0.018).

Anthropometric factors and smoking status also were associated with longitudinal changes in biomarker concentrations. For instance, for waist circumference the baseline (interleukin-6) and serial increase (interleukin-6, P-selectin) were associated with higher inflammatory markers at follow-up. Similarly, both baseline (CRP and TNFRII) and serial increases (CRP, MCP-1, and TNFRII) in body-mass index also were associated with increasing concentrations in biomarkers. Smoking at the baseline examination was associated with increased concentration of many of the biomarkers (CRP, interleukin-6, sICAM-1, isoprostanes, Lp-PLA2-mass, and osteoprotegerin) at follow-up.

Lipid concentrations and lipid treatment were associated with longitudinal changes in several biomarkers. Baseline (sICAM-1, Lp-PLA2 activity), and increasing total/HDL ratio (CRP, sICAM-1, Lp-PLA2 activity and mass, P-selectin and TNFRII) or higher baseline triglycerides (P-selectin) were associated with increasing concentrations of the biomarkers at follow-up. Conversely, lipid-lowering treatment either at the index examination or on follow-up was associated with decreased concentration of CRP and Lp-PLA2 activity and mass (Table 4).

A few risk factors were only associated with one or two biomarker levels. For instance, baseline and change in diastolic blood pressure were associated with declining TNFRII concentrations. Similarly, baseline and starting hormone replacement therapy were associated with increasing mean TNFRII concentrations. New diabetes was associated with increasing osteoprotegerin concentrations.

Despite the cross-sectional associations with isoprostanes, interleukin-6 and TNFRII (eTable 3), heavy alcohol consumption, and antihypertensive treatment were not associated with change in biomarkers. Prevalent CVD and aspirin use also were not associated with change in inflammatory biomarker concentrations.

The adjusted *R*-squared depicts the amount of variability in the change in biomarker concentrations explained by baseline biomarker levels and baseline and change in clinical risk factors. We observed that the clinical risk factors explained a moderate amount of variability of the change in biomarker concentrations between two consecutive examinations. Change in TNFRII over follow-up had the least amount of variability explained by the clinical factors ($r^2 = 0.280$), and serial Lp-PLA2-mass had the highest ($r^2 = 0.52$).

4. Discussion

In our large community-based sample we observed the following main findings. First, not surprisingly, for each biomarker, baseline inflammatory biomarker concentration explained the largest amount of variability in longitudinal change in its level. The baseline concentration was the only factor associated with serial change in every biomarker. Mean baseline concentrations were negatively associated with biomarker change. Second, older age at baseline was associated with increased follow-up concentration in the majority of biomarkers (CRP, interleukin-6, sICAM-1, MCP-1, osteoprotegerin and isoprostanes). Third, anthropometric factors were associated with progressive increases in mean inflammatory biomarker concentrations including baseline body-mass index (CRP, TNFRII) and waist circumference (interleukin-6), as well as serial increases in body-mass index (CRP, MCP-1 and TNFRII) and increase in waist circumference (interleukin-6, P-selectin). Fourth, smoking at the baseline examination was associated with longitudinal increases in more than half of the biomarkers (CRP, interleukin-6, sICAM-1, isoprostanes, Lp-PLA2-mass, and osteoprotegerin). Fifth, except for MCP-1 and osteoprotegerin, high lipid levels (or worsening of lipid profile on follow-up) also were commonly associated with increasing concentrations of biomarkers over time, as opposed to lipid medications that were associated with declining concentrations of biomarkers. Finally, CVD risk factors explained only a moderate amount of the longitudinal change in a panel of inflammatory biomarkers.

By exponentiating beta coefficients from multivariable models, we can estimate the expected change of the ratio of biomarker concentration of follow-up over the baseline concentration. For example, for CRP, corresponding with analysis of raw data (not shown), holding all the clinical covariates constant (assuming no change in clinical covariates statistically associated with change of CRP), participants who stopped hormone replacement therapy would have an estimated relative ~30% decrease in CRP concentrations, relative to individuals who did not stop hormone replacement therapy. Similarly, participants who were smokers at baseline had a ~35% increase in CRP concentrations, as compared to individuals who were not smokers, assuming no change in other clinical covariates.

As shown in Table 3, we observed weak-to-modest correlations of baseline levels of biomarkers and change in concentration of biomarkers on follow-up. A priori, we expected only weak correlations between biomarkers at baseline, as we selected the biomarkers to

represent different biological pathways. Interestingly, the lack of a stronger correlation of change in biomarkers over time suggests that factors associated with change of inflammation are specific to each individual marker, which is further supported by the data in Table 3.

There are several potential explanations for the observed lower concentrations of several biomarkers on follow-up examination. One possible explanation is that there was a substantial increase in medical therapies for hypertension, hypercholesterolemia and prevention of CVD with aspirin from our index examination to the follow-up examination. Participants with higher concentrations of biomarkers at baseline had higher risk profiles, thus were more likely to receive new or more intensive drug therapies. Another potential explanation for lower concentrations on follow-up is regression to the mean.

The inflammatory biomarkers we report have been related to incident CVD events. For instance, CRP, interleukin-6, sICAM-1, isoprostanes and P-selectin have been related to new-onset MI; Lp-PLA2 and osteoprotegerin have been related to incident heart failure; CRP has been related to incident stroke, and CRP, sICAM-1 and osteoprotegerin have been associated with incident atrial fibrillation and progression of peripheral arterial disease [7, 17–30]. Whereas prior reports have focused on relating CVD risk factors cross-sectionally to inflammatory biomarkers, we sought to examine the relation of changing risk factors to progression of systemic inflammation. Identifying elements that promote systemic inflammation may offer insights into the development of CVD, thus suggesting targets for CVD prevention.

Despite the low prevalence of smoking behavior in our cohort, a central finding of our study is that smoking behavior is fundamentally associated with systemic inflammation [31, 32]. Cigarette smoking was associated with inflammatory biomarkers cross-sectionally at both examinations (all biomarkers except TNFRII, and MCP-1 at the follow-up examination, eTable 3). Similarly smoking at baseline was associated with longitudinal increases in most of the biomarkers (all but Lp-PLA2 activity, MCP-1, P-selectin and TNFRII). Although our data are observational and only 2% of participants stopped smoking between the two consecutive examinations, the associations suggest that one beneficial mechanism of smoking cessation may be declining levels of systemic inflammation.

Cross-sectional and longitudinal human and experimental data have consistently demonstrated that measures of adiposity are associated with inflammatory biomarkers [33–38]. Considering the obesity epidemic, the associations between weight gain and inflammation are concerning. Weight gain, reflected by both increases in body-mass index or increases in waist circumference, was associated with rising concentrations in multiple inflammatory biomarkers.

Our data are in line with literature suggesting that worsening of lipid profile is associated with increased burden of inflammation and the treatment with lipid-lowering medications is associated with lower concentrations of inflammatory biomarkers. For instance, experimental data show that mice fed with high-fat diets have higher levels of P-selectin and Lp-PLA2-mass [39, 40]. Large observational and experimental studies have suggested that the presence of hyperlipidemia is associated with inflammation and atherosclerosis [41, 42]. Recent interventional trials have shown the effect of lipid-lowering medications on biomarkers of inflammation, suggesting a causal link between lipid treatment and reduction of inflammation [43].

There are several possible explanations as to why our data reflected that blood pressure measures had little association with progression of inflammatory biomarkers. We only observed that baseline and increasing diastolic blood pressure were associated with

declining TNFRII concentrations. It should be noted that the longitudinal change in blood pressure was very mild (a mean increase of 2–3 mmHg, Table 1). In addition, the relation of blood pressure to change in concentration of biomarkers may have been accounted for by other clinical factors in the stepwise exclusion model. The associations of increase in blood pressure with higher inflammation may have been counterbalanced by the increase in hypertension treatment between the two examinations. It is also possible that blood pressure and hypertension treatment are not associated with increased inflammation; however, this is less likely given cross-sectional and longitudinal data supporting such a relation [44, 45].

Whereas clinical factors explained between 28% (TNFRII) and 52% (Lp-PLA2-mass) of the variability in inflammatory markers, much of the variability in cross-sectional and longitudinal changes in systemic inflammation remains unexplained. There are many potential contributors to the unexplained variability in measures of systemic inflammation. We acknowledge that measurement variability may play a role. In addition, although we examined baseline and change in multiple clinical covariates, there are additional factors we did not include in our models, such as physical activity and psychosocial factors. The substantial unexplained variation in inflammatory marker concentrations suggests that there are individual and unique components in the development of CVD. Focusing on unknown factors related to inflammation may lead to the discovery of modifiable factors of inflammation and CVD incidence. Finally, we and other investigators have noted that genetic factors contribute to some of the inter-individual variation in systemic inflammatory markers [15, 46].

Cardiovascular risk factors have been associated cross-sectionally with concentrations of inflammatory biomarkers in many prior publications [33, 47–50], which we confirmed in our cross-sectional evaluations of the two consecutive examination cycles (eTable 2). Although our group and others have examined serial CRP measurements, to the best of our knowledge, this is the largest longitudinal study of factors associated with the progression of multiple inflammatory biomarkers in a community-based cohort [8, 51]. Additionally, the study was conducted with routinely and rigorously ascertained biomarkers and clinical risk factors.

The present study has several limitations. We cannot infer causal relations between the covariates and the change in biomarkers given the observational nature of the study. Second, due to the numerous covariates and biomarkers examined in our study, we acknowledge multiple statistical testing, which we addressed by employing a Bonferroni correction for a *p*-value of 0.0001 (0.05 divided by 15 covariates). Third, because we needed to account for multiple testing, we also acknowledge that in a larger data set, additional clinical covariates may have been associated with longitudinal change in inflammatory biomarkers for all but isoprostanes we had 80% power to detect a true partial correlation of 0.076 or larger in absolute value between clinical variables with change in ln(biomarker). Fourth, the time interval between examinations (~7 years) was intermediate and we measured the panel of biomarkers on only two occasions; we may have failed to detect factors associated with change in systemic inflammation over shorter or longer time frames. Fifth, concerns regarding the generalizability of the data must be acknowledged. Our sample was middle-aged to elderly at baseline; we may not be able to extrapolate these findings to other age groups. The small sample size, the mixed ethnic/racial composition of the Omni Cohort and the non-fasting collection on the first examination cycle markedly limited our ability to identify the relation between ethnic/racial variation and longitudinal changes in inflammatory biomarkers. The different patterns of change in concentration of inflammatory biomarkers between the Omni and Offspring Cohorts may reflect the difference in blood collection on the Omni's baseline examination, rather than true biological effects. Given the small sample sizes, ethnic/racial variation, and differences in technique, we acknowledge

our Omni data are hypothesis generating. Further studies are necessary to clarify the relations between ethnic/racial variation and change in biomarkers over time.

In our longitudinal community-based study, we describe the clinical covariates associated with change in inflammatory biomarkers over follow-up. Given the potential for the use of serial biomarker measurements in clinical practice, it is imperative to appreciate the factors involved with their change. Thus, it is important to highlight that traditional clinical risk factors explained a moderate amount of change in the inflammatory biomarkers. The relations between cigarette smoking, lipids and lipid therapies, and anthropometry and longitudinal progression of systemic inflammation provide indirect support for preventive cardiovascular risk factor modification. However, much of the variability in biomarker concentrations remains unexplained. Further studies are necessary to enhance our knowledge of the mechanisms responsible for, and therapies that will effectively protect against, the progression of systemic inflammation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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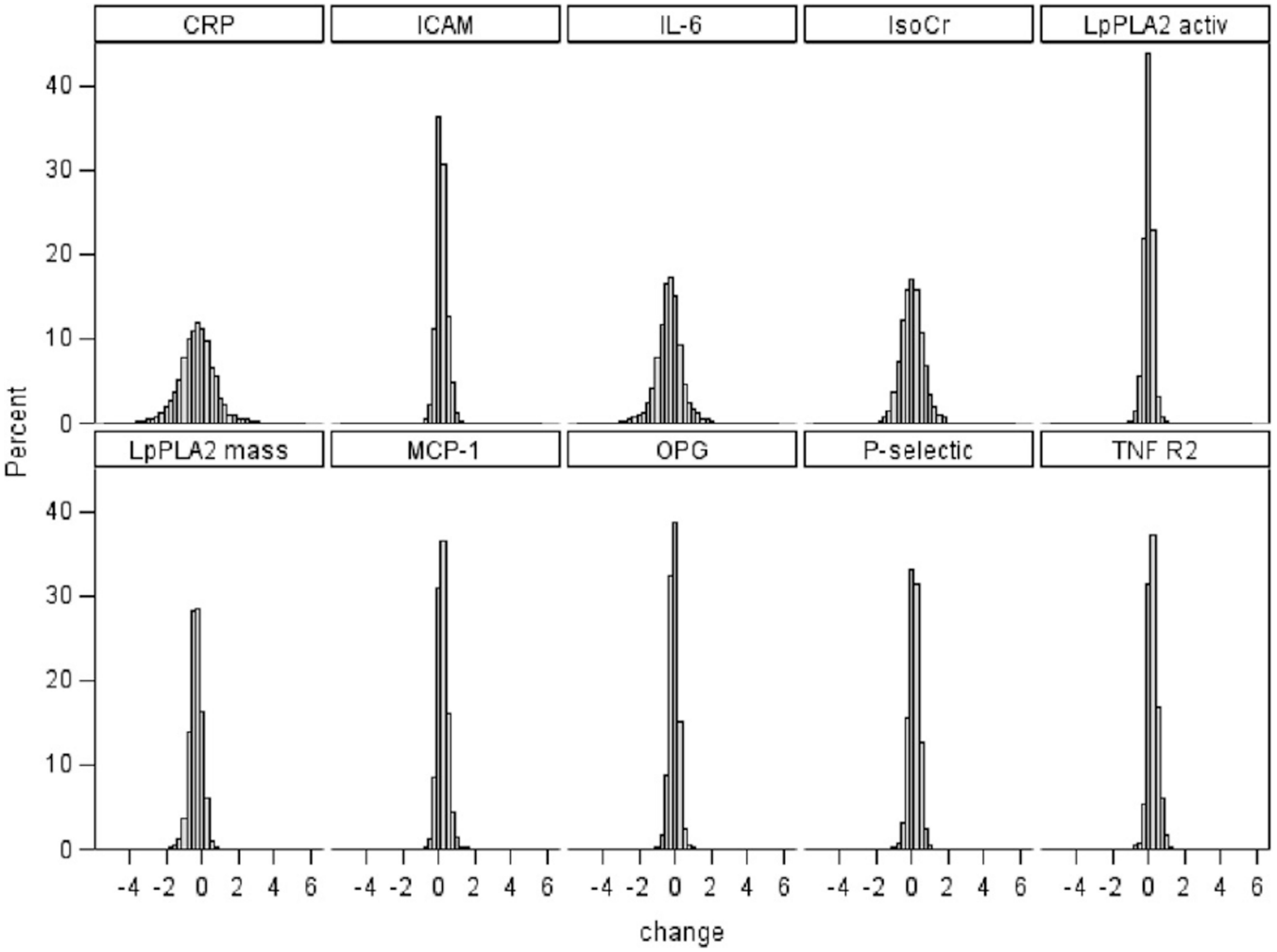

Fig. 1.
Histogram for changes in log biomarkers.

Table 1

Participant characteristics.

	Men, *n* = 1353		Women, *n* = 1660	
	[a]Baseline	[b]Follow-up	[a]Baseline	[b]Follow-up
Age, y	59 ± 9	66 ± 9	59 ± 9	66 ± 9
Cigarette smoking, %	155(11)	111(8)	196(12)	146(9)
Systolic blood pressure, mmHg	127 ± 16	129 ± 17	125 ± 19	128 ± 18
Diastolic blood pressure, mmHg	76 ± 9	75 ± 10	73 ± 10	72 ± 10
Body-mass index, kg/m^2	28.8 ± 4.6	28.9 ± 4.8	27.6 ± 5.8	27.8 ± 5.9
Waist circumference, in	40 ± 5	41 ± 5	38 ± 6	39 ± 6
Total/HDL cholesterol ratio	4.5 ± 1.3	3.7 ± 1.1	3.6 ± 1.2	3.3 ± 1.0
Triglycerides, mg/dL	142 ± 95	119 ± 76	130 ± 81	116 ± 65
Glucose, mg/dL	107 ± 28	110 ± 26	99 ± 24	103 ± 21
Diabetes, %	187(14)	245(18)	152(9)	190(11)
Hypertension treatment, %	456(34)	718(53)	453(27)	755(46)
Lipid-lowering medication, %	313(23)	665(49)	256(15)	627(38)
Hormone replacement therapy, %			525(32)	159(10)
Aspirin treatment[c], %	509(38)	714(53)	370(22)	591(36)
Prevalent cardiovascular disease,%	97(7)	145(11)	39(2)	71(4)

Data are presented as mean ± standard deviation, or *n*(%).

[a]Baseline examination is Offspring 7 and Omni 2.

[b]Follow-up examination represents Offspring 8 and Omni 3.

[c]Aspirin treatment ≥3 times week.

Table 2

Summary for median concentration of biomarkers at baseline and follow-up examination.

Variable	Baseline			Follow-up		
	Median	Q1	Q3	Median	Q1	Q3
CRP, mg/L	2.04	0.95	4.88	1.49	0.74	3.21
sICAM-1, ng/mL	238	207	279	271	230	336
Interleukin-6, pg/mL	2.54	1.72	4.09	1.77	1.18	2.87
Isoprostanes, pg/mmol	130	88	190	128	96	178
Lp-PLA2 activity, nmol/mL/min	138	116	163	136	114	160
Lp-PLA2-mass, ng/mL	278	223	351	200	170	229
MCP-1, pg/mL	305	244	375	364	299	441
Osteoprotegerin, pmol/L	5.21	4.31	6.22	4.72	3.90	5.70
P-Selectin, ng/mL	36.0	28.4	45.0	39.7	32.6	47.9
TNFRII, pg/mL	1918	1609	2310	2368	1913	3029

Table 3

Correlation between change in log concentration of biomarkers.

Change in log biomarker concentration	Change in log biomarker concentration									
	CRP	**Interleukin-6**	**sICAM-1**	**Isoprostanes**	**Lp-PLA2-mass**	**Lp-PLA2 activity**	**MCP-1**	**Osteoprotegerin**	**P-selectin**	**TNFR2**
CRP	1.00									
Interleukin-6	0.43†	1.00								
sICAM-1	0.12†	0.05*	1.00							
Isoprostanes	0.02	0.01	0.07†	1.00						
Lp-PLA2-mass	0.06*	0.03	0.04*	0.01	1.00					
Lp-PLA2 activity	0.05*	0.01	0.06*	−0.03	0.55†	1.00				
MCP-1	0.03	0.10†	0.12†	0.05*	0.05*	0.08†	1.00			
Osteoprotegerin	0.06†	0.05*	0.02	0.04	0.02	−0.03	0.01	1.00		
P-selectin	0.14†	0.10†	0.05*	0.05*	0.10†	0.07†	0.09†	0.01	1.00	
TNFR2	0.08†	0.05*	0.12†	0.05*	0.00	0.02	0.05*	−0.05*	0.04*	1.00

Notes:

*p-value between 0.05 and 0.001;

†p-value less than 0.001.

Table 4

Clinical covariates of change in biomarkers concentration: results of 2-stage stepwise modeling.

Covariates	**CRP**		**sICAM-1**		**Interleukin-6**		**Isoprostanes**		**Lp-PLA2 activity**	
	β	**SE**	**β**	**SE**	**β**	**SE**	**β**	**SE**	**β**	**SE**
Biomarker value, baseline	−0.494	0.018	−0.464	0.020	−0.544	0.017	−0.736	0.015	−0.493	0.018
Age, baseline	0.102	0.017	0.063	0.016	0.213	0.017				
Omni Cohort			−0.377	0.060	0.287	0.062	−0.187	0.046	−0.190	0.049
Interaction Omni* baseline			0.341	0.052						
Sex (female)			0.127	0.032			0.234	0.028		
Total/HDL baseline			0.091	0.021					0.356	0.019
Change Total/HDL	0.077	0.019	0.099	0.020					0.589	0.022
Smoke, baseline	0.305	0.050	0.213	0.050	0.376	0.051	0.29	0.045		
Body-mass index, baseline	0.131	0.018								
Change in body-mass index	0.200	0.017								
Lipid therapy, baseline	−0.272	0.044								
Lipid therapy, start	−0.212	0.044							−0.321	0.037
Hormone replacement therapy, baseline										
Hormone replacement therapy, start										
Hormone replacement therapy, stop	−0.281	0.051								
Diastolic blood pressure, baseline										
Change in diastolic blood pressure										
Waist Circumference, baseline					0.147	0.017				
Change in Waist Circumference					0.107	0.016				
Triglycerides, baseline										
Change triglycerides									−0.164	0.017
Diabetes, new-onset										
Adjusted *R*-squared	0.298		0.304		0.285		0.515		0.466	
	Lp-PLA2-mass		MCP-1		Osteoprotegeri		P-selectin		TNFR2	
	Est β	SE	Est β	SE	Est β	SE	Est β	SE	Est β	SE
Biomarker value, baseline	−0.705	0.014	−0.539	0.016	−0.475	0.017	−0.515	0.016	−0.193	0.010

Covariates	CRP		sICAM-1		Interleukin-6		Isoprostanes		Lp-PLA2 activity	
	β	SE	β	SE	β	SE	β	SE	β	SE
Age, baseline			0.064	0.016	0.278	0.018			0.12	0.011
Interaction age*baseline					0.095	0.016				
Omni Cohort					0.315	0.057				
Interaction Omni*baseline	0.317	0.061								
Sex (female)										
Total/HDL, baseline										
Change Total/HDL	0.145	0.016					0.092	0.019	0.045	0.010
Smoke, baseline	0.269	0.048			0.196	0.050				
Body-mass index, baseline									0.044	0.010
Change in body-mass index			0.078	0.015					0.040	0.010
Lipid therapy, baseline	−0.296	0.037								
Start lipid therapy	−0.320	0.035								
Hormone replacement therapy, baseline									0.095	0.024
Hormone replacement therapy Start									0.109	0.026
Hormone replacement therapy, Stop										
Diastolic blood pressure, baseline									−0.053	0.012
Change in diastolic blood pressure									−0.089	0.011
Waist circumference, baseline										
Change in Waist circumference							0.077	0.015		
Triglyceride, baseline	0.061	0.014								
Change in triglyceride							0.092	0.022		
Diabetes, new-onset					0.324	0.051				
Adjusted *R*-squared	0.520		0.297		0.311		0.309		0.280	