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Long-Term Variability of Inflammatory Markers and Associated Factors in a Population-Based Cohort

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Abstract

OBJECTIVES—To investigate long-term variability in serum high sensitivity C-reactive protein (CRP) and interleukin-6 (IL-6) and to determine associated risk factors for high risk inflammatory profiles.

DESIGN—Prospective population-based cohort study.

PARTICIPANTS—Participants (N=1,443) of the Epidemiology of Hearing Loss Study and the Beaver Dam Eye Study, two population-based prospective studies of aging in the same cohort.

MEASUREMENTS—Among participants aged 43–79 years at the initial exam (1988–1990), serum high sensitivity CRP was measured from three time-points (1988–1990, 1998–2000, 2009–2010) and serum IL-6 was measured from two (1998–2000 and 2009–2010).

RESULTS—When IL-6 levels were categorized into tertiles, 50.8% of participants remained in the same group 10 years later (weighted Kappa(κ)=.34). When CRP was categorized into three risk groups, 53.4% of participants remained in the same group during 10 years (κ =.36), and 32.4% remained in the same group at all three examinations (κ =.27). Interleukin 6 increased from a geometric mean of 1.54 pg/L to 1.78 pg/L over 10 years, while CRP increased from a geometric mean of 1.67 mg/L to 2.25 mg/L over 10 years, and then decreased to 1.93 mg/L over the next 10 years. These 10-year decreases in CRP were not observed in those not reporting statin use. Factors

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associated with long-term higher levels of either IL-6 and CRP included older age (IL-6), obesity, smoking, lower physical activity (IL-6), lower HDL cholesterol (IL-6), and a history of statin (non)use (CRP).

CONCLUSION—Inflammatory marker levels tracked over the long-term into older age with within-person increases observed. Several potentially modifiable risk factors were associated with long-term higher levels of inflammatory markers.

Keywords

Inflammation; Aging; Long-term Variability

INTRODUCTION

Epidemiological studies are increasingly focusing on the role of inflammation in the development of chronic diseases associated with aging. Two common markers of systemic inflammation are serum interleukin-6 (IL-6) and C-reactive protein (CRP). IL-6 is a pro-inflammatory cytokine produced by many different cell types including white blood cells, endothelial cells, and adipose cells and is involved in a wide range of functions throughout the body.¹ Once activated, IL-6 also mediates the acute phase inflammatory response inducing the release of CRP from hepatic cells.² CRP is one of a number of acute-phase proteins that make up the nonspecific physiological response to tissue damage and infection.² In older adults, higher serum levels of both IL-6 and CRP are associated with age-related diseases such as cardiovascular disease,^{3,4} Alzheimer's disease,^{5,6} and type II diabetes.⁷

Aging is often thought to be accompanied by a chronic low-grade pro-inflammatory state.^{8,9} Indeed, numerous cross-sectional studies have demonstrated that levels of many pro-inflammatory markers are higher in older adults than younger adults.^{5,10} Yet, to date, few longitudinal studies have demonstrated how these markers change within individuals over a long period of time. The aims of this study were to characterize changes in IL-6 and CRP in older adults and to identify potential modifiable risk factors for increased inflammatory burden with age.

METHODS

The Epidemiology of Hearing Loss Study (EHLS) began in 1993 to measure hearing endpoints in an existing population-based cohort developed for the Beaver Dam Eye Study (BDES).^{11,12} Both studies examined the same participants concurrently at the same examination site every 5 years, with a high retention rate among living participants (> 80%) for each phase. Cohort and follow-up characteristics for the BDES1 (1988–1990) and EHLS2 (1998–2000) examinations have been published previously.^{11–13} As expected, in a longitudinal study of older adults, participants in EHLS2 were younger and had a higher socioeconomic status than non-participants.¹³ Participants in this current study include those with inflammatory marker data from both the EHLS2 and the EHLS4 (2009–2010) examinations. Mean time between measurements was 10.6 years (range: 9.0 to 12.8 years). To further assess the representativeness of this study population, these participants (N=1,443) were compared with EHLS2 participants still alive at the time of EHLS4, but not included in this study (N=452) either due to nonparticipation or missing inflammation data. Study participants were younger (mean difference = 2.8 years ($P<0.0001$), and more likely to be male (42.5% vs. 37.2% $P=0.045$). Study participants and living nonparticipants did not differ significantly by educational status or EHLS2 inflammatory marker levels (results not shown).

Non-fasting venous blood was drawn at EHLS2 and EHLS4, processed at the time of the draw, and stored in a -80C freezer following similar protocols. In 2011, serum IL-6 was measured (Advanced Research and Diagnostic Laboratory, University of Minnesota, Minneapolis, MN) on each participant's serum sample from EHLS2 and EHLS4 using the quantitative sandwich enzyme technique of ELISA (QuantiKine High Sensitivity kit, R&D Systems, Minneapolis, MN). All samples were paired and assayed on the same plate to eliminate between-run variability. Commercially obtained controls and in-house controls were run daily with an inter-assay coefficient of variation (CV) of 11.7%. Blinded replicate samples for both EHLS2 (n=44) and EHLS4 (n=33) were included with the samples. For both phases of the study, repeatability was high (IL-6 Spearman $r = 0.95$).

Serum high-sensitivity CRP assays at all time-points were performed at the same laboratory using a latex-particle enhanced immunoturbidimetric assay and in accordance with strict quality control procedures. The first round of high-sensitivity CRP testing was performed in 2006 from samples drawn at BDES1 (1988–1990) and stored in -80 C for up to 16.5 years with one previous freeze-thaw cycle.¹⁴ The assay (Kamiya Biomedical Company, Seattle, WA) was performed on the Roche/Hitachi 911 Analyzer (Roche Diagnostics, Indianapolis, IN). High sensitivity CRP was determined on samples from EHLS2 and EHLS4 in 2011 using the assay from Roche Diagnostics on the Roche Modular P Chemistry Analyzer. The reference range for both methods for the laboratory was 0 – 5 mg/L, and the laboratory inter-assay CV's were 4.5% for both methods. Blinded replicate samples for EHLS2 (n=44) and EHLS4 (n=33) showed that repeatability for high sensitivity CRP was high (Spearman $r = 0.99$). In order to compare long-term with short-term within-person variability, inflammatory markers were also assayed in a small convenience sample of participants (n=41) seen twice at EHLS2 over an average of 10 months (range: 1 to 24 months).

At each phase of EHLS/BDES trained examiners administered extensive questionnaires. The number of years of education a participant had obtained was divided into 3 groups: less than 12 years, 12 years, and greater than 12 years of education. Participants were grouped by history of smoking into one of three groups: never smoker (fewer than 100 cigarettes lifetime), past smoker (smoked at least 100 cigarettes, but stopped prior to exam), and current smoker (more than 100 cigarettes, and still currently smoking). Participants self-reported the number of various alcohol beverages consumed (beer, wine, liquor) in an average week which was then converted into total grams of ethanol per week, and categorized as per previously defined cutoffs.¹⁵ A weekly total physical activity index was created which summed kilocalories from the number of city blocks walked (1 block per day = 56 kcal/wk) and stairs climbed (1 flight per day = 28 kcal/wk).¹⁶ A history of cardiovascular disease (CVD) was defined as self-report of physician diagnosed stroke, myocardial infarction, or angina. Data on all medications used in the last month were collected via labels on medications brought in during the exam or by self-report.

Height and weight was measured and body mass index (BMI) was calculated by dividing weight in kilograms by height in meters squared. Participants were considered overweight if their BMI was greater than or equal to 25 kg/m² but less than 30 kg/m², and obese if their BMI was greater than or equal to 30 kg/m². Diabetes was defined as either a self-report of physician diagnosis of diabetes, taking medications for diabetes (pills or insulin injection), or hemoglobin A1C level greater than or equal to 6.5% at the time of the exam. Blood pressure was measured with Random Zero Sphygmomanometers using the Hypertension Detection and Follow-up Protocol, 1976. Hypertension was defined as systolic blood pressure greater than or equal to 140 mmHg, diastolic blood pressure greater than or equal to 90 mmHg, or physician-diagnosed hypertension and current use of hypertension medication. Serum total and high-density lipoprotein (HDL) cholesterol were measured using colorimetric spectrometry (Vitros Analyzer, Beaver Dam Community Hospital Laboratory).

Statistical Analysis

For categorical analysis, participants were divided into three CRP risk groups (<1, 1–3, >3 mg/L) based on established cut-points¹⁷ and three IL-6 groups according to tertiles of the distribution at EHLS2 (<1.07, 1.07–2.01, >2.01 pg/L) since risk group cut-points have yet to be established. Long-term within-group stability was determined by percent agreement and Kappa statistics.

Short- and long-term variability of inflammatory markers was compared after the removal of outliers using the Cochran's c test which uses the ratio of the maximum variance to the sum of variances.¹⁸ A random effects analysis of variance was then performed using Proc Mixed (subject as random variable) in SAS (SAS Institute, Cary, NC). Intraclass correlation (ICC) was used as a measure of reliability, calculated as the between-person variance component divided by the between-person plus the within and analytic variance components. The number of samples needed to reach an ICC of 0.75 (considered excellent reliability) was calculated using the Spearman-Brown Formula.¹⁹

Interleukin-6 and CRP data were log transformed due to skewness and the geometric means and 95% confidence intervals (CI) were reported for the various phases of the study by age group and sex. The difference over time was determined by subtracting the earlier logged level from the later logged level. Symmetric percentage (s%) change was then calculated by multiplying the log difference by 100, as defined by Cole (2000) and was interpreted as a percent change on the log scale.²⁰ Inflammation change (on the natural log scale) was regressed on age and sex to statistically test differences.

Since studies have shown the importance of both increases and sustained high-levels of inflammatory markers in the development of chronic diseases,^{3,21–23} logistic regression was used to determine predictors of a long-term higher-risk inflammatory profile defined as either increasing a level or remaining in the highest level of inflammation over 10-years (EHLS2 and EHLS4). To construct model 2, those factors suggestively associated ($P < .25$) with the outcome in model 1 (age- and sex-adjusted) were added, and then the model was refined using log-likelihood ratio tests. Final analyses further examined the effects of long-term statin use and non-steroidal anti-inflammatory drug (NSAID) (including aspirin) use on the results of this study. All analyses were conducted using SAS 9.2.

RESULTS

Between the EHLS2 (1998–2000) and EHLS4 (2009–2010) examinations, 50.8% of participants remained within the same IL-6 group (weighted Kappa (κ) = .34) (Table 1). Over this time-period 29.3% increased at least one IL-6 level while 19.9% decreased at least one level. This level of variability was similar by sex (data not shown), but in older age groups was somewhat increased (Equality of κ between age groups: $P = 0.021$). Over the same time-period 53.4% ($\kappa = .36$) stayed within the same CRP group. A greater percentage of participants decreased (28.8%) by at least 1 level than increased (17.9%). In those participants with CRP measured over 20 years ($n = 1,438$), nearly one third of participants (32.4%) remained in the same group at all three exam phases ($\kappa = .27$), and of participants in the high risk group (>3 mg/L) at baseline ($n = 393$), 44.0% remained in that group at both the 10- and 20-year follow-up exams. When compared to CRP levels at BDES, percent agreement was similar for both 10- and 20-year follow-up (51.5% and 50.6% respectively) (Table 2). Long-term variability (10- and 20-year) for CRP was similar across age groups or sex (data not shown). Long-term categorical percent agreement (10-year and 20-year) was similar after removing participants with CRP levels indicating a possible acute infection at any study phase (>10 mg/L) (10-year: $N = 198$; 20-year: $N = 229$) (data not shown). Excluding

participants using statin drugs at any time, the 10- and 20-year percent agreement improved for CRP (58.6%, 36.7%) groups.

Over the short-term, levels did not differ for IL-6 (mean difference: 0.06 pg/mL (95%CI -0.12, 0.23 pg/mL) or CRP (mean difference: 0.02 mg/L (95%CI -0.20, 0.24 mg/L) in a subsample (IL6: N=38; CRP: N=39) of participants with measurements taken on average 10 months apart. Long-term variation (10-years) in both IL-6 and CRP was substantially greater than short-term variation (10 months). For both IL-6 and CRP, the ICCs were considerably greater for short-term variability (0.72, 0.80 respectively) than for long-term variability (0.53, 0.49 respectively). In order to reach a level of reliability considered excellent (ICC=.75) 1 sample was needed to represent a participant's level over the short-term but 3 were needed over the long term for each marker.

Between EHLS2 and EHLS4, levels of IL-6 increased for 57.0% of participants and decreased for 42.8% participants. Overall IL-6 increased 14.8s% (95%CI: 10.6s%, 18.9s%) over 10 years (median increase: 0.23 pg/mL (5th percentile: +0.05, 95th percentile +1.73 pg/mL)) (Table 3). Ten year increases were successively higher for each age group (P for trend < 0.0001; sex-adjusted), reaching 52.0s% (95%CI: 26.3s%, 77.7s%) in participants aged 80–89 years at EHLS2. Over this same time-period CRP levels increased in 43.0% of participants and decreased in 56.6% of participants for an overall 10-year decrease of 15.6s% (95%CI: -21.2s%, -10.0s%) (median decrease: -0.40 mg/L (5th percentile: -0.07 mg/L, 95th percentile -0.52 mg/L)). When data were added from BDES (1988–1990), 10 years prior, CRP levels increased over the first 10 years 30.1s% (95%CI: 25.0s%, 35.3s%) and then decreased over the next 10 years, for an overall 20-year increase of 14.5 s% (95%CI: 8.7s%, 20.4s%). Twenty-year increases in CRP were largest in younger participants, and were fairly stable in the oldest age groups (P for trend across age groups=0.001; sex adjusted).

From EHLS2 to EHLS4, history of statin use increased from 20.7% to 48.6%, while NSAID use increased from 47.3% to 71.8%. When stratified by longitudinal statin use categories (never user, new user at EHLS4, continual user at both EHLS2 and EHLS4), 10-year decreases in CRP were statistically significant for new (P<0.0001) and continual users (P<0.0001), but not for never users (P=0.463) (Figure 1A). Final levels (EHLS4) of CRP were statistically significantly higher in statin never users than in new and continual users after adjusting for age and sex. CRP levels at EHLS4 did not statistically differ by NSAID use category (Figure 1B) although trends were similar to those of statin use. Neither EHLS2 nor EHLS4 IL-6 levels differed statistically by statin use or NSAID use category (Figure 1C&D). When medication use (NSAID and statins) was categorized by duration of use using data from all waves of EHLS, results were similar to those reported figures 1A–D for both CRP and IL-6. For example 10-year decreases in CRP were statistically significant for short term (>0 to <12.5years use) statin users (P<0.0001) and long-term (≥ 12.5 years) statin users (P<0.0001), but not for never users (P=0.766).

Factors independently associated with having a higher-risk IL-6 profile over 10 years included older age, current smoking (versus never smoking), physical activity (≥ 561 kCal/wk vs. <197 kCal/wk), obesity (BMI ≥ 30 kg/m² vs. < 25 kg/m²), and lower levels of HDL cholesterol (42–54 mg/dL vs. < 42 mg/dL) (Table 4). Current smoking and obesity were also associated with higher-risk CRP profile, as was higher HDL cholesterol (≥ 55 mg/dL vs. < 42 mg/dL). Conversely, statin users were less likely to have a higher-risk CRP profile (OR vs. nonusers: 0.73, 95%CI: 0.55, 0.96). In a sensitivity analysis after removing participants using statins, higher levels of HDL were no longer statistically significantly associated with a higher risk CRP profile (OR for ≥ 55 mg/dL vs. < 42 mg/dL: 1.25 95%CI: 0.88, 1.77). In further sensitivity analyses, point estimates for both IL-6 and CRP final models were similar

after the removal of participants with acute CRP values ($>10\text{mg/L}$) at EHLS2 or EHLS4 ($N=198$),¹⁷ and after the removal of participants with a chronic inflammatory condition ($N=232$) defined as a self-report of emphysema, gout, or asthma (data not shown).

DISCUSSION

As research develops on the complex relationship between systemic inflammation and diseases associated with aging, it will be important to understand the characteristics and associated factors of various inflammatory markers over the life-course. This is one of the first studies to represent long-term measures of inflammatory markers in older adults. There was considerable 10-year consistency within groups for both IL-6 and CRP. Within-person IL-6 levels increased over a period of 10 years and CRP levels increased over a period of 20 years, supporting the possibility that inflammatory dysregulation occurs with age. Trajectories of CRP over time differed considerably by statin and NSAID use, suggesting that medication use should be considered in future analyses of inflammatory markers. Several modifiable factors were associated with long-term, higher-risk inflammatory profiles including smoking, physical activity, and obesity.

Previous studies have demonstrated that IL-6 and CRP are fairly stable markers over the short-term and suitable for use in epidemiological studies.^{24,25} Fewer studies have been published on longer-term variability of these markers in larger populations, especially for IL-6, and fewer still have taken medication use into account. Although it was somewhat difficult to compare across studies where inflammatory groups were defined differently, in this study the 10-year percent agreement between groups for IL-6 (50.8%) and CRP (53.4%) were in line with stability estimates in studies of shorter duration,^{26,27} and with a study done using measures 16 years apart in the Framingham cohort.²⁸ In the current study, nearly a third of participants remained in the same CRP group when 3 CRP measures across 20-years were used. Ten and 20-year stability of categorical CRP levels improved when statin users were removed (58.6%, 36.7% respectively). The results from these longer time-frames suggest a considerable degree of tracking into older age for both IL-6 and CRP.

It is commonly reported that IL-6 levels are higher in older adults; however, most of the evidence comes from cross-sectional studies.^{5,8,29} In EHLS, within-person IL-6 levels increased longitudinally 14.8 s% (median increase of 0.23 pg/mL) over 10 years, with the largest increases observed in the oldest participants. Longitudinal increases in IL-6 (+0.9 pg/mL in women and +0.8 pg/mL in men) were also seen in the Cardiovascular Health Study All Stars Study over a period of 9 years in participants over 72 years of age at baseline.²² An increasing age-associated low-grade inflammatory state, sometimes termed inflammaging,³⁰ is thought to contribute to the pathogenesis of various diseases in older adults.^{9,31,32} Increased inflammation with age has been attributed to increasing morbidity and cardiovascular risk factors which accompany older age, as well as other age-associated processes such as declining levels of sex hormones, and increasing oxidative damage or cellular stress.^{8,9,31,33}

C-reactive protein levels have also been shown to be minimally increased in older participants in cross-sectional studies.¹⁰ In this study, within-person CRP increased over 10 (30.1s%) and 20 (14.5s%) years, with a decrease observed over the last 10 years. Stable or decreasing CRP has been reported previously in a longitudinal study involving older participants.²² Differences between these longitudinal studies and previous cross-sectional studies may be due to differences in population structure, or due to increased medication use in older adults. In clinical trials statin use was associated with decreasing CRP levels in both the short and long term.^{34,35} These results have been supported by cross-sectional population-based studies, where statin use was associated with lower CRP levels.^{36–38}

Given the increased prevalence of statin drug usage, population-wide CRP levels may be decreasing along with decreasing cholesterol levels.³⁹ When longitudinal changes in CRP were stratified by statin use, 10-year decreases were observed in continual and new users of statins, but not in non-users. Similar trends were observed for NSAID use in this study, although results from previous studies on the effects of NSAIDs on CRP have been quite variable.^{40, 41} These data provide longitudinal evidence that statin and NSAID use may have an effect on CRP levels over the long-term in the general population. IL-6 levels did not differ by statin and NSAID use, which may explain the different longitudinal trends observed for these two inflammatory markers.

Several previously determined risk factors for higher-levels of systemic inflammation were also found to be associated with long-term inflammatory marker levels in this study. Obesity and smoking were significant predictors of a higher-risk IL-6 and CRP profile over 10 years, while physical activity was associated with lower odds of a high-risk IL-6 profile. The association between BMI and markers of inflammation has been demonstrated often in a wide variety of populations in several longitudinal studies ranging from 3 to 16 years.^{27,28,42} Similarly, smoking has also shown to be associated with higher levels of circulating inflammatory markers in a wide-range of studies.^{38,43} An inflammatory mechanism has been proposed for linking obesity and smoking with chronic disease (CVD, diabetes); these results support this hypothesis.^{43,44} Although mechanisms underlying the cardio-protective effect of physical activity are still being determined, reductions in systemic inflammation levels with physical activity may play a role.⁴⁵ Other cohort studies of aging have shown that increased physical activity is associated with lower levels of inflammation often independent of body composition,^{45,46} which was the case with IL-6 in this study as well. These results show that several potentially modifiable factors were associated with increased long-term inflammatory marker levels.

While HDL has been shown to be cardio-protective, less data are available on its association with circulating cytokines and acute-phase proteins.^{47,48} In this study, higher HDL was associated with a higher-risk CRP profile. These results are difficult to interpret given the high prevalence of statin use in this cohort, and the fact that statin use may have had an effect on both HDL and CRP levels. When statin users were removed from the analyses, high levels of HDL cholesterol were no longer statistically significantly associated with a CRP inflammation profile. More data are needed on the relationships between HDL cholesterol and inflammation levels over time especially in light of the wide-spread increased prevalence of statin use.

The aims of this study were achieved by using a large, well-characterized longitudinal epidemiological cohort with a wide age-range and inflammatory markers measured over 10 years for IL-6 and 20 years for CRP. Several limitations need to be noted. To be included in this study, participants needed to survive to both the 1998–2000 and 2009–2010 examinations and therefore sample size was restricted for adults over age 90 years at the 2009–2010 examination. However this study included participants unable to come to the clinic including those home-bound or living in nursing homes which helped to reduce losses to follow-up in the oldest old. Participants with complete data over the course of this study were most likely healthier than those lost to follow-up through death, which may explain in part why associations were not observed between chronic diseases such as CVD and hypertension and long-term inflammatory levels in this cohort. However, baseline 1998–2000 inflammatory marker levels were not different between participants who returned for the 2009–2010 examination, and living participants who did not which suggests that the results of this study fairly represent the inflammatory profiles of the living members of this cohort. Although BDES1 CRP was assayed at an earlier time, the same laboratory performed each measurement using the same methodology, and assay variability was

similar. Inflammatory marker data from the 1998–2000 and 2009–2010 were assayed at the same time to keep analytic variability to a minimum.

This study provided longitudinal data showing that inflammation levels tended to track into older age, and that to some extent, levels increased overtime within individuals. Several potentially modifiable risk factors were associated with a long-term higher-risk inflammatory profile including smoking, obesity, low levels of physical activity and the (non)use of statins. Further research is needed to more fully understand long-term trajectories of systemic inflammation, possibly through the use life-course approaches with more frequent measurement of markers, over longer time-frames, and to understand the relationships between these trajectories and the development of chronic diseases of aging.

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Figure 1. Least-Squared Geometric Mean (95%CI) CRP (mg/L) and IL-6 (pg/L) Levels at 1998–2000 and 2009–2010 by 10-year Statin and NSAID Use Category, Adjusted for Age and Sex in the Epidemiology of Hearing Loss Study.
A. CRP levels by statin use category; B. IL-6 levels by statin use category; C. CRP levels by NSAID use category; D. IL-6 levels by NSAID use

Table 1

Ten-year Agreement (%) between IL-6 Levels and CRP Levels at Epidemiology of Hearing Loss Study2 (1998–2000) and EHLS4 (2008–2010) in 1,443 Participants with Paired Data

<u>EHLS2 IL-6</u>	<u>EHLS4 IL-6</u>			<u>EHLS2 CRP</u>	<u>EHLS4 CRP</u>		
	Low (N=380)	Medium (N=495)	High (N=568)		Low (N=408)	Medium (N=580)	High (N=455)
Low (N=480)	16.2	10.7	6.4	Low (N= 302)	13.1	5.8	2.0
Medium (N=478)	7.0	13.9	12.3	Medium (N=596)	10.5	20.8	10.1
High (N=485)	3.1	9.8	20.7	High (N=545)	4.7	13.6	19.5

IL-6 Tertiles: Low (<1.07 pg/mL), Medium (1.07–2.01 pg/mL), High (>2.01 pg/mL); CRP levels: Low (<1 mg/L), Medium (1–3 mg/L), High (>3 mg/L).

Note: Cell percentages may not sum to 100% due to rounding

CRP=C-reactive Protein; EHLS=Epidemiology of Hearing Loss Study; IL-6=Interleukin-6

Table 2

Ten- and Twenty-year Agreement (%) between CRP levels at Beaver Dam Eye Study (1988–1990), and Epidemiology of Hearing Loss Study2 (1998–2000) and EHLS 4 (2008–2010) in 1,438 Participants with Paired Data

BDES CRP	EHLS2 CRP			Subject (n)	EHLS4 CRP		
	<1 mg/L	1–3 mg/L	>3 mg/L		<1 mg/L	1–3 mg/L	>3 mg/L
<1 mg/L	14.0	13.3	5.1	465	15.9	11.5	5.0
1–3 mg/L	4.7	20.2	15.4	580	8.6	20.0	11.8
>3 mg/L	2.1	7.9	17.4	393	3.7	8.8	14.8
Subjects (n)	299	594	545	1438	405	579	454

Note: Cell percentages may not sum to 100% due to rounding

BDES=Beaver Dam Eye Study; CRP=C-reactive Protein; EHLS=Epidemiology of Hearing Loss Study; IL-6=Interleukin-6

Table 3

Geometric Means (95% Confidence Intervals (CI)) and Symmetric Percent Change Over Time for CRP and IL-6 at Various Phases of the Beaver Dam Eye Study and the Epidemiology of Hearing Loss Study

		BDES1 (1988–1990)	EHLS2 (1998–2000)	EHLS4 (2009–2010)	10 –Year[†]	20-Year[†]
	N	Geometric Mean (95%CI)	Geometric Mean (95%CI)	Geometric Mean (95%CI)	Symmetric Percent Change (95%CI)	Symmetric Percent Change (95%CI)
IL-6 (pg/L)						
Overall	1443	--	1.54 (1.47, 1.60)	1.78 (1.71, 1.86)	14.8 (10.6, 18.9)	--
Women	830	--	1.59 (1.51, 1.68)	1.77 (1.68, 1.88)	10.8 (5.5,16.1)	--
Men	613	--	1.46 (1.38, 1.55)	1.79 (1.67, 1.91)	20.1 (13.6,26.6)	--
Baseline Age Group						
53–59 y	456	--	1.38 (1.29, 1.49)	1.47 (1.37, 1.58)	6.2 (–0.9, 13.2)	--
60–69 y	615	--	1.61 (1.50, 1.72)	1.76 (1.65, 1.88)	9.2 (2.7, 15.7)	--
70–79 y	328	--	1.60 (1.49, 1.72)	2.21 (2.04, 2.40)	32.1 (25.0, 40.2)	--
80–89 y	44	--	1.73 (1.41, 2.12)	2.91 (2.16, 3.91)	52.0 (26.3, 77.7)	--
CRP (mg/L)						
Overall	1438	1.67 (1.58, 1.76)	2.25 (2.13, 2.38)	1.93 (1.82, 2.04)	30.1 (25.0, 35.3)	14.5 (8.7, 20.4)
Women	828	1.77 (1.65, 1.91)	2.63 (2.45, 2.83)	1.98 (1.83, 2.13)	39.4 (32.7, 46.2)	10.9 (3.1, 18.8)
Men	610	1.53 (1.42, 1.65)	1.82 (1.69, 1.97)	1.86 (1.71, 2.02)	17.5 (9.6, 25.4)	19.4 (10.6, 28.2)
Baseline Age Group						
43–49 y	460	1.49 (1.35, 1.65)	2.21 (1.99, 2.44)	1.99 (1.80, 2.20)	39.0 (29.4, 48.7)	28.7 (18.5, 39.0)
50–59 y	612	1.64 (1.52, 1.77)	2.32 (2.14, 2.51)	1.82 (1.67, 1.99)	34.7 (27.4, 42.0)	10.8 (2.1, 19.4)
60–69 y	322	1.94 (1.74, 2.17)	2.22 (1.98, 2.49)	2.02 (1.79, 2.28)	13.3 (1.9, 24.7)	4.0 (–9.4, 17.4)
70–79 y	44	2.15 (1.57, 2.96)	2.10 (1.53, 2.86)	2.06 (1.44, 2.95)	–2.8 (–27.1, 21.5)	–4.4 (–38.5, 29.6)

Note: Symmetric Percent change (s%) = $100 \times (\log_e \text{EHLS4} - \log_e \text{EHLS2})$ (Cole 2000)

BDES=Beaver Dam Eye Study; CRP=C-reactive Protein; EHLS=Epidemiology of Hearing Loss Study; IL-6=Interleukin-6

[†]Symmetric percent change from BDES1 (1988–1990)

Table 4

Odds Ratios (OR) and 95% Confidence Intervals (CI) for High-risk Inflammation Levels over 10 years in the Epidemiology of Hearing Loss Study (1998/2000 – 2009/2010).

Characteristics (1998–2000)	N	IL-6		CRP	
		Model 1[†]	Model 2[†]	Model 1[†]	Model 2[†]
Age, 5yr	1443	1.30 (1.21, 1.40)	1.35 (1.25, 1.46)	0.97 (0.90, 1.04)	1.01 (0.94, 1.09)
Male Sex	613	1.11 (0.90, 1.38)	0.97 (0.75, 1.25)	1.05 (0.85, 1.31)	1.03 (0.80, 1.33)
Education, years					
<12	182	Ref	--	Ref	--
12	729	0.88 (0.62, 1.23)	--	0.89 (0.63, 1.24)	--
>12	531	0.73 (0.51, 1.04)	--	0.70 (0.49, 1.00)	--
Smoking					
Never	677	Ref	Ref	Ref	Ref
Past	600	1.26 (1.00, 1.59)	1.19 (0.93, 1.51)	1.37 (1.08, 1.73)	1.32 (1.03, 1.68)
Current	137	1.89 (1.29, 2.76)	2.03 (1.36, 3.02)	1.48 (1.02, 2.17)	1.55 (1.05, 2.29)
Physical Activity, kCal/wk					
Low (0–196)	504	Ref	Ref	Ref	--
Med (197–560)	452	0.66 (0.51, 0.85)	0.66 (0.51, 0.87)	0.91 (0.70, 1.18)	--
High (561+)	467	0.60 (0.46, 0.78)	0.69 (0.52, 0.91)	0.79 (0.60, 1.03)	--
Alcohol consumption (g/wk)					
1–14	629	Ref	--	Ref	--
Non-drinker	241	1.34 (0.99, 1.82)	--	1.28 (0.94, 1.73)	--
15–75	246	0.80 (0.59, 1.08)	--	0.98 (0.72, 1.34)	--
75–140	151	1.03 (0.72, 1.49)	--	1.41 (0.98, 2.04)	--
140+	147	1.31 (0.90, 1.92)	--	1.33 (0.91, 1.95)	--
BMI, kg/m ²					
< 25	223	Ref	Ref	Ref	Ref
25–30	485	1.29 (0.92, 1.80)	1.32 (0.93, 1.87)	1.04 (0.73, 1.47)	1.18 (0.82, 1.68)
30	679	2.05 (1.49, 2.82)	1.95 (1.39, 2.76)	1.71 (1.24, 2.38)	2.04 (1.44, 2.89)
HDL cholesterol, mg/dL					
Low (<42)	443	Ref	Ref	Ref	Ref
Medium (42–54)	488	0.71 (0.54, 0.93)	0.75 (0.57, 0.99)	0.84 (0.64, 1.11)	0.86 (0.65, 1.15)
High (55)	469	0.63 (0.47, 0.84)	0.75 (0.55, 1.03)	1.16 (0.87, 1.55)	1.39 (1.02, 1.90)
Total cholesterol, mg/dL					
Low(<197)	465	Ref	--	Ref	--
Medium (197–226)	481	1.11(0.85, 1.44)	--	1.02(0.78, 1.33)	--
High (227)	488	0.90 (0.69, 1.17)	--	1.13 (0.87, 1.48)	--
Chronic Disease					
Diabetes	147	1.24 (0.87, 1.76)	--	1.26 (0.89, 1.78)	--
CVD	149	1.11 (0.78, 1.59)	--	1.13 (0.79, 1.61)	--
Hypertension	765	1.14 (0.92, 1.42)	--	1.30 (1.05, 1.62)	--
Medication Use					

Characteristics (1998–2000)	N	<u>IL-6</u>		<u>CRP</u>	
		Model 1 [†]	Model 2 [†]	Model 1 [†]	Model 2 [†]
Statins	294	1.03 (0.79, 1.34)	--	0.75 (0.57, 0.99)	0.73 (0.55, 0.96)
NSAIDs	673	1.16 (0.94, 1.44)	--	0.97 (0.78, 1.20)	--
Vitamin D supplement	729	0.81 (0.65, 1.01)	--	0.76 (0.61, 0.94)	--
Hormones (women)	274	0.82 (0.60, 1.12)	--	0.78 (0.57, 1.08)	--

Note: High-risk Inflammation levels defined as either increasing a level or remaining in the highest level of inflammation over 10-years.

BMI=Body Mass index; CRP=C-reactive Protein; CVD=Cardiovascular disease; HDL=High-density Lipoprotein; IL-6=Interleukin-6; NSAIDs=Non-steroidal Anti-inflammatory Drugs

[†]Model 1 controlling age and sex; Model 2 controlling for sex, age, and other reported predictors in the model. Sample size for individual risk factors may not sum to 1443 due to missing covariate data.