



## Prospective study of IL-18 and risk of MI and stroke in men and women aged 60–79 years: A nested case-control study

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### ABSTRACT

**Aim:** IL-18 is hypothesized to destabilise atherosclerotic plaques, leading to thrombotic events and epidemiologic studies suggest that IL-18 may increase risk of CHD or CVD. We examined prospective associations between levels of serum IL-18 and new CHD and stroke events in older men and women from a general population.

**Methods:** A case-control study was nested within a prospective cohort of men and women aged 60–79 years recruited from general practices in 25 British towns in 1998–2000 and followed-up for 7.5 years for fatal and non-fatal MI and stroke. Baseline IL-18 was measured in stored serum samples of incident cases of MI ( $n = 364$ ) or stroke ( $n = 300$ ) and two controls per case.

**Results:** Geometric mean IL-18 levels were higher among the 364 MI cases than the 706 controls; 417.84 pg/mL (IQR 316.25, 537.44) compared to 386.90 pg/mL (IQR 296.54, 482.33),  $p(\text{difference}) = 0.002$ . IL-18 was positively associated with adverse lipid and inflammatory profiles. Men and women in the top third of baseline IL-18 levels had an age and sex-adjusted odds ratio (OR) for MI of 1.31 (95%CI 0.92, 1.85) compared with those in the lowest third; this attenuated to 1.05 (95%CI 0.72, 1.53) after additional adjustment for established vascular and inflammatory risk factors. Each doubling of IL-18 level was associated with an increased OR for MI 1.34 (95%CI 1.04, 1.72), which was attenuated on adjustment for established vascular and inflammatory risk factors; 1.09 (95%CI 0.83, 1.44). Geometric mean IL-18 levels did not differ between stroke cases and controls. The OR for stroke associated with the highest compared to the lowest tertile of IL-18 was 1.24 (95%CI 0.84, 1.84). Results for MI and stroke did not differ by presence of pre-existing CVD, gender or age.

**Conclusions:** Circulating IL-18 levels were strongly associated with a range of established and novel risk factors but were not independently associated with risk of MI or stroke in our study.

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### 1. Introduction

Inflammation plays an important role in atherosclerosis and several acute phase reactant proteins are associated with elevated risk of cardiovascular disease (CVD). Interleukin 18 (IL-18) is a pro-inflammatory cytokine which is highly expressed in atherosclerotic plaques (mainly in plaque macrophages) and is detected in higher levels in unstable than stable plaques [1]. IL-18 is thought to contribute to destabilisation of atherosclerotic plaques, leading to

thrombotic events [1,2]. Plaque rupture is important in both CHD and in the commonest type of stroke in older persons, atherothrombotic stroke. We recently conducted a meta-analysis of twelve prospective epidemiologic studies of IL-18 and onset of coronary heart disease (CHD) or CVD and found that higher levels of IL-18 were associated with onset of CHD: participants in the top third of baseline IL-18 levels had approximately 64% increased risk of CHD, which attenuated to 34% after additional adjustment for established risk factors and inflammatory markers [3]. Associations did not differ appreciably between IL-18 and CHD alone or combined endpoints including CVD.

To date most studies of IL-18 and CHD or stroke are of middle aged adults, only three of the studies in the meta-analysis were of populations with an average age over 65 years at study entry.

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Extrapolating findings to older adults may be problematic; as the importance of established risk factors such as blood pressure and cholesterol are attenuated at older age [4,5], the relative importance of inflammatory markers in CVD aetiology may be heightened. Alongside possible changes in the aetiology of CVD in older age groups, levels of circulating inflammatory markers increase with age, as do levels of subclinical vascular disease and the number of co-morbidities associated with inflammation. Studies of composite CVD endpoints including stroke together with other CHD events find evidence that IL-18 is associated with raised risk of CVD [6,7] but fewer studies have specifically investigated the role of IL-18 in the onset of stroke. Three studies including patients from high risk groups (with history of stroke or CVD risk factors), did not find evidence that IL-18 was associated with risk of stroke [8–10].

We therefore examined prospective associations between IL-18 and MI and stroke in a case-control study of older men and women, aged 60–79 years at baseline, nested in two cohorts followed-up for up to 8.5 years for incident CVD events. We examined whether IL-18 was independently associated with MI or stroke in men and women in the general population, and also whether the associations varied by age, gender and presence of pre-existing CVD. We also examined whether any associations were mediated by other inflammatory markers implicated in CVD.

## 2. Materials and methods

In 1998–2000, 4252 men from a single General Practice in each of 24 British towns who were already taking part in a prospective study of cardiovascular disease attended for follow-up measurements at the age of 60–79 years (response rate 77%) [11]. In 1999–2001, a parallel study of 4286 women of the same age and in the same Practices was established, with the addition one more town (Bristol), response rate 60% [12]. All subjects provided written informed consent to the investigation and ethical approval was provided by relevant local research ethics committees. Near identical study protocols were used. In both studies, nurses administered questionnaires, and women self-completed a separate additional detailed questionnaire. Pre-existing CVD was defined from validated reports from General Practitioner, or self-report of MI or stroke at any questionnaire between 1978–80 and 1998–2000 in men and in the baseline questionnaire (1999/2001) in women [12]. Data on cigarette smoking, alcohol consumption, physical activity, own longest-held occupation (or husband's occupation for non-married women, coded using the Registrar General's classification) were self-reported. Region of residence was recorded for women in 1998–2001 and for men at the start of their follow-up in 1978–80. Nurses made physical measurements (weight, height, seated blood pressure and FEV<sub>1</sub>), recorded an ECG and collected fasting venous blood samples, from which serum was stored at  $\leq -70^\circ\text{C}$  for subsequent analysis of cholesterol, triglycerides, haemostatic and inflammatory markers as described elsewhere [11–14]. IL-18 (pg/mL) was measured from stored frozen serum samples by researchers blinded to case control status using enzyme-linked immunosorbent assays (R&D systems, Abingdon, UK) according to the manufacturer's instructions. Inter- and intra-assay coefficients of variation were 10.4% and 5.6%. IL-18 was measured twice in 158 subjects over a 4 year interval, in 1996 and 2000 to enable intra-individual comparisons of values over time [15].

Participants were followed-up for 6.25–8.5 years for mortality and cardiovascular morbidity, with a follow up loss of <2%. Fatal cases were ascertained through National Health Service Central Registers from a death certificate: ICD-9 codes 410–414 for MI and ICD-9 codes 430–438 for stroke, indicating deaths with cerebrovascular disease as the underlying cause. Non-fatal MI or stroke

was diagnosed using World Health Organisation diagnostic criteria [16] based on validated reports from General Practitioners, supplemented by regular reviews of General Practice records [11,12].

We established a nested case-control study based on all 394 CHD cases occurring between February 1998 and June 2006 in men and between April 1999 and September 2007 in women. A total of 788 controls "frequency matched" to cases on town of residence, gender and age in 5-year bands were randomly selected from among subjects who had survived to the end of the study period free from incident CHD. Similarly a separate nested case control study in 324 cases of stroke and 648 controls was established. In the sample with complete data ( $n = 895$ ), there were 299 MI cases, from which we would have had 80% power to detect a relative risk of 1.67 in the top tertile of IL-18 compared to the bottom tertile, at the 5% statistical significance level. From 235 cases of stroke, similarly we would have had 80% power to detect a relative risk of 1.79, assuming 2 controls per case.

### 2.1. Statistical analyses

Highly skewed variables were natural log transformed as necessary. Means and standard deviations of baseline characteristics of cases and controls aged 60–79 years were calculated. Continuous variables were adjusted for gender, baseline region of residence and age at examination. Distributions of categorical variables were examined. Tertiles of IL-18 were defined in the MI control sample. Associations between IL-18 tertiles and covariates were examined using one way ANOVA. Established risk factors were selected *a priori* and the novel inflammatory and haemostatic risk markers were selected on the basis that they had been reported elsewhere to be associated with MI and stroke. Unmatched logistic regression analyses were used to examine associations between IL-18 tertiles and MI. Models were adjusted for (i) gender, age and region of residence, (ii) established CHD risk factors fitted as continuous variables (BMI, systolic and diastolic blood pressure, triglycerides, total and HDL cholesterol) and the following categorical variables; smoking ("current", "ex-smoker" or "never smoker"), alcohol use ("1–2 drinks/day" or "other") physical activity ("more than 3 h of moderate/vigorous activity per week" or "less"), history of diabetes ("present" or "absent") (iii) novel risk factors (natural log transformed CRP and IL-6). Linear regression was used to model the association between risk of MI and continuous IL-18 (log<sub>2</sub> transformation) in order to assess the effect of a doubling of IL-18 level on risk of MI. Differences in the IL-18-MI or stroke associations were tested using likelihood ratio (LR) tests for interactions with age, gender and pre-existing CVD (MI or stroke). Rosner's method was used to calculate regression dilution ratios on a subset of men with repeated IL-18 measurements, using a linear regression model of the repeat measurement predicted by the first measurement [17].

## 3. Results

### 3.1. MI

IL-18 data were available for 364 of 394 MI cases and for 706 of 788 controls. Cases were aged on average 70.9 years, predominantly male (73%) and 45% were in non-manual occupations (Table 1). Cases differed from controls in having a higher prevalence of pre-existing CVD and diabetes, more current smokers, fewer drinkers of 1–2 units of alcohol/day and had lower levels of physical activity (Table 1, all  $p < 0.02$ ). Cases had higher SBP and lower FEV<sub>1</sub> but similar BMI and DBP to controls. Cases had less favorable lipid, inflammatory and metabolic marker profiles. Geometric mean IL-18 levels were higher among the 364 MI cases

**Table 1**Baseline characteristics (age 60–79) of the MI case and control populations: mean (SD) unless otherwise specified<sup>a</sup>.

	MI cases (n = 364)	MI controls (n = 706)	Difference (p value)
<i>Demographic/questionnaire</i>			
Age (years)	70.87 (5.47)	70.83 (5.47)	Matched
Male, n(%)	267 (73.4)	512 (72.5)	Matched
Northern region of residence, n(%) <sup>b</sup>	151 (41.5)	291 (41.2)	Matched
Non-manual occupation, n(%) <sup>b</sup>	154 (44.7)	323 (47.9)	0.351
Prior evidence of CVD, n(%)	109 (30.0)	119 (16.9)	<0.001
History of diabetes, n(%)	66 (18.1)	75 (10.6)	0.001
1–2 alcoholic drinks/day, n(%)	127 (37.9)	301 (46.2)	0.013
Current smoker, n(%)	75 (20.6)	94 (13.3)	0.002
Physical activity (inactive/occasional), n(%)	233 (66.6)	397 (58.8)	0.016
<i>Physical measurements</i>			
Body Mass Index (kg/m <sup>2</sup> ) <sup>c,e</sup>	27.11 (4.26)	27.03 (3.97)	0.833
Systolic blood pressure (mm/Hg) <sup>c,d,e</sup>	153.84 (27.37)	148.58 (24.09)	0.001
Diastolic blood pressure (mm/Hg) <sup>c,d,e</sup>	83.75 (12.14)	83.22 (11.50)	0.482
FEV <sub>1</sub> (L/min) <sup>c,e,g</sup>	2.15 (0.55)	2.23 (0.60)	0.032
<i>Lipids/metabolic markers</i>			
Total cholesterol (mMol/L)	6.25 (1.23)	6.08 (1.07)	0.020
HDL cholesterol (mMol/L)	1.32 (0.33)	1.42 (0.36)	<0.001
Triglyceride (mMol/L) <sup>c,d,f</sup>	1.80 (1.31, 2.39)	1.58 (1.13, 2.09)	<0.001
Insulin (mU/L) <sup>c,d,f</sup>	9.54 (6.04, 13.97)	8.14 (5.57, 11.23)	<0.001
Glucose (mMol/L) <sup>c,d,f</sup>	6.18 (5.35, 6.39)	5.82 (5.31, 6.11)	<0.001
<i>Inflammatory and hemostatic markers</i>			
IL-18(pg/mL) <sup>c,f</sup>	417.84 (316.25, 537.44)	386.90 (296.54, 482.33)	0.002
C-Reactive Protein (mg/L) <sup>c,f</sup>	2.41 (1.06, 5.53)	1.77 (0.89, 3.60)	<0.001
IL-6(pg/ml) <sup>c,d,f</sup>	2.99 (1.93, 4.08)	2.43 (1.61, 3.44)	<0.001
Fibrinogen(g/L) <sup>c,d,f</sup>	3.46 (3.04, 3.95)	3.27 (2.85, 3.77)	<0.001
White cell count ( $\times 10^9/L$ ) <sup>c,d,f</sup>	7.28 (6.02, 8.74)	6.81 (5.67, 7.97)	<0.001

<sup>a</sup> Case-control sample is maximum available.<sup>b</sup> Reported in 1978–1980 (age 40–59 years) for men and in 1998–2000 (age 60–79 years) for women.<sup>c</sup> Adjusted for gender, age at survey and region of residence (Scotland, North, Midlands and South).<sup>d</sup> Adjusted for time of day.<sup>e</sup> Adjusted for nurse number.<sup>f</sup> Analysed as natural log transformed variable, geometric mean (IQR) reported on original scale.<sup>g</sup> Adjusted for height squared.

than the 706 controls; 418 pg/mL (IQR 316, 537) compared to 387 pg/mL (IQR 297, 482),  $p$ (no difference) = 0.002 (Table 1).

### 3.2. Stroke

IL-18 data were available for 300 of 324 cases and 590 of 648 controls. Cases were aged on average 71.3 years, were predominantly male (64%) (Table 2). Cases differed with respect to controls in having higher prevalence of pre-existing CVD and more smokers ( $p < 0.002$ ). Cases had higher blood pressure and lower FEV<sub>1</sub> but similar BMI. Cases had similar lipid profile, except for lower total cholesterol. Cases had higher IL-6 and white blood cell count, but they were similar to controls in respect to other inflammatory and haemostatic markers (Table 2). Geometric mean IL-18 levels did not differ significantly between the 300 cases and the 590 controls; 406 pg/mL (IQR 311, 532) compared to 389 pg/mL (IQR 300, 492)  $p$ (no difference) = 0.13 (Table 2).

### 3.3. Baseline correlates of IL-18

Table 3 summarizes the associations between IL-18 and cardiovascular risk factors across thirds of the MI control population (the larger control population). Associations in the stroke control population were similar (Web Table 1), unless noted below. IL-18 levels were higher in men and in subjects with diabetes but did not vary with age, social or behavioral factors, FEV<sub>1</sub>, BP or total cholesterol. IL-18 was positively associated with BMI, triglycerides and inversely associated with HDL cholesterol. Correlations between log IL-18 and covariates were all  $r < 0.25$  (e.g. in the MI control population  $r = -0.23$  for HDL  $p < 0.001$ ;  $r = 0.20$  for log insulin,  $p < 0.05$ ;  $r = 0.16$  for t-PA  $p < 0.001$ ;  $r = 0.13$  for log CRP,  $p = 0.02$  and  $r = 0.10$

for log IL-6,  $p = 0.20$ ). Weak inverse associations with social class and positive associations with cigarette smoking, insulin and glucose did not reach conventional levels of statistical significance in the MI control population, but did in the stroke control population. IL-18 was positively associated with CRP, IL-6, fibrinogen, plasma viscosity, Fibrin D-dimer, Von Willebrand Factor and tissue plasminogen activator. Positive associations with white blood cell count and Factor VIII only reached statistical significance in the stroke control sample.

### 3.4. Associations between IL-18 and MI

Table 4 summarizes the associations between MI and tertiles of IL-18. In Model 1 (adjusted for gender, age and region of residence), the odds ratio (OR) for MI associated with the highest compared to the lowest tertile of IL-18 ( $T_3$  vs  $T_1$ ) was 1.31 (95%CI 0.92, 1.85) and there was no strong statistical evidence of a linear trend in ORs across the three groups ( $p = 0.13$ ). The ORs were attenuated on adjustment for established risk factors (Model 2, smoking, alcohol, physical activity, history of diabetes, BMI, systolic and diastolic blood pressure, total and HDL cholesterol):  $T_3$  vs  $T_1$ : 1.09 (95%CI 0.75, 1.58). Further adjustment for CRP and IL-6 (Model 3) had a small attenuating effect:  $T_3$  vs  $T_1$  OR 1.05 (95%CI 0.72, 1.53). Each doubling of IL-18 level was associated with increased risk of MI (Model 1); 1.34 (95%CI 1.04, 1.72), however, this was attenuated to the null on adjustment for established risk factors and other inflammatory markers CRP and IL-6 (Model 3); 1.09 (95%CI 0.83, 1.44).

We conducted a sensitivity analysis to see whether the associations differed in participants without any pre-existing MI or stroke compared to those with pre-existing disease. In stratified

**Table 2**  
Baseline characteristics (age 60–79) of the stroke case and control populations: mean (SD) unless otherwise specified<sup>a</sup>.

	Stroke cases (n = 300)	Stroke controls (n = 590)	Difference (p value)
<i>Demographic/questionnaire</i>			
Age (years)	71.32 (5.33)	71.34 (5.26)	Matched
Male, n(%)	191 (63.7)	372 (63.1)	Matched
Northern region, n(%) <sup>b</sup>	107 (35.7)	221 (37.5)	Matched
Non-manual occupation, n(%) <sup>b</sup>	123 (46.4)	259 (46.9)	0.679
Prior evidence of CVD, n(%)	84 (28.0)	88 (14.9)	<0.001
History of diabetes, n(%)	42 (14.0)	72 (12.2)	0.448
1–2 alcoholic drinks/day, n(%)	105 (38.5)	210 (38.3)	0.953
Current smoker, n(%)	52 (17.3)	57 (9.7)	0.002
Physical activity (inactive/occasional), n(%)	196 (70.8)	380 (67.0)	0.273
<i>Physical measurements</i>			
Body Mass Index (kg/m <sup>2</sup> ) <sup>c,e</sup>	27.06 (4.36)	27.11 (4.09)	0.875
Systolic blood pressure (mm/Hg) <sup>c,d,e</sup>	154.19 (25.07)	149.18 (24.23)	0.004
Diastolic blood pressure (mm/Hg) <sup>c,d,e</sup>	84.80 (12.59)	82.35 (11.18)	0.003
FEV <sub>1</sub> (L/min) <sup>c,e,g</sup>	2.09 (0.57)	2.23 (0.53)	<0.001
<i>Lipids/metabolic markers</i>			
Total cholesterol (mMol/L)	6.13 (1.22)	6.31 (1.17)	0.025
HDL cholesterol (mMol/L)	1.42 (0.39)	1.42 (0.36)	0.839
Triglyceride (mMol/L) <sup>c,d,f</sup>	1.65 (1.11, 2.32)	1.69 (1.23, 2.29)	0.473
Insulin (mU/L) <sup>c,d,f</sup>	8.88 (5.62, 12.99)	8.26 (5.23, 11.58)	0.136
Glucose (mMol/L) <sup>c,d,f</sup>	6.03 (5.29, 6.17)	5.87 (5.29, 6.11)	0.095
<i>Inflammatory and hemostatic markers</i>			
IL-18 (pg/ml) <sup>c,f</sup>	406.34 (310.50, 531.77)	389.20 (300.17, 491.64)	0.131
C-Reactive Protein (mg/L) <sup>c,f</sup>	2.12 (1.04, 4.28)	1.92 (0.91, 4.16)	0.217
IL-6 (pg/ml) <sup>c,d,f</sup>	2.85 (1.88, 4.13)	2.57 (1.65, 3.55)	0.028
Fibrinogen (g/L) <sup>c,d,f</sup>	3.35 (2.95, 3.84)	3.28 (2.88, 3.70)	0.139
White cell count ( $\times 10^9/L$ ) <sup>c,d,f</sup>	7.24 (6.12, 8.57)	6.91 (5.79, 8.22)	0.017

<sup>a</sup> Case control sample is maximum available n = 890.

<sup>b</sup> Reported in 1978–1980 (age 40–59 years) for men and in 1998–2000 (age 60–79 years) for women.

<sup>c</sup> Adjusted for gender, age at survey and region of residence (Scotland, North, Midlands and South).

<sup>d</sup> Adjusted for time of day.

<sup>e</sup> Adjusted for nurse number.

<sup>f</sup> Analysed as natural log transformed variable, geometric mean (IQR) reported on original scale.

<sup>g</sup> Adjusted for height squared.

analyses, we did not observe any associations between IL-18 and MI (Table 4). A likelihood ratio (LR) test for interaction found no evidence for difference between the two groups,  $p = 0.63$ . Gender differences in associations between tertiles of IL-18 and MI were tested and none were observed (LR test  $p = 0.67$ ). Furthermore, there was no evidence that the association between IL-18 and MI varied by age (LR test  $p = 0.99$ ).

### 3.5. Associations between IL-18 and stroke

Table 5 summarizes the associations between tertiles of IL-18 and stroke, in Model 1 (adjusted for gender, age and region of residence), the OR for stroke associated with the highest compared to the lowest tertile of IL-18 T<sub>3</sub> vs T<sub>1</sub> was 1.24 (95%CI 0.84, 1.84) and there was no strong evidence of a linear trend across the three groups ( $p = 0.29$ ). There was no strong evidence of an association between continuous IL-18 and stroke; the OR for a doubling of IL-18 was 1.26 (95%CI 0.84, 0.89), Model 1. A sensitivity analysis stratified by presence of prior CHD or stroke (Table 5), confirmed the results of the main analysis; no associations between IL-18 and stroke were observed in either the sample with pre-existing disease or without pre-existing disease, LR test for interaction,  $p = 0.38$ . Gender differences in associations between tertiles of IL-18 and stroke were tested and none were observed (LR test  $p = 0.84$ ). Additionally, there was no evidence that the association between IL-18 and stroke varied by age (LR test  $p = 0.54$ ).

### 3.6. Regression dilution

Using Rosner's method a regression dilution ratio of 0.71 (95%CI 0.61, 0.81) for IL-18 was calculated in a subset of 158 men with

IL-18 levels measured 4 years apart [17]. The OR of MI among men in the top third of IL-18 compared with the lowest third, adjusted for age and vascular risk factors (Model 3) 1.09 (95%CI 0.75, 1.58), was 1.13 (95%CI 0.67, 1.90) after correction for regression dilution. The equivalent OR for strokes were 1.07 (95%CI 0.71, 1.61) and 1.10 (95%CI 0.62, 1.96) after correction for regression dilution.

## 4. Discussion

In this nested case-control study, set within a prospective population-based cohort of 60–79 year old women and men, higher serum IL-18 levels were associated with higher levels of other inflammatory markers (CRP, IL-6, fibrinogen and white cell count), hemostatic markers (plasma viscosity, factor VIII, fibrin D-dimer, Von Willebrand factor and t-PA), with established cardiovascular risk factors lipids (blood pressure and lipids) and also health behaviors (cigarette smoking, participating in low levels of physical activity and being a non-drinker or a heavy drinker). Baseline IL-18 levels were not associated with elevated risk of MI at conventional levels of statistical significance, consistent with some but not all previous studies. The absence of association between IL-18 and stroke over the follow-up period is in keeping with existing, albeit sparse literature about IL-18 and stroke.

### 4.1. Strengths and weaknesses

The study benefits from validated data about MI and stroke events and has comprehensive data on a wide range of established and novel cardiovascular risk factors. However, we were unable to distinguish between subtypes of stroke, although we expect the

**Table 3**Association between IL-18 (tertiles) and cardiovascular risk factors in the MI control sample (*n* = 706).

	Low (95–324 pg/ml) <i>n</i> = 239	Medium (325–450 pg/ml) <i>n</i> = 232	High (451–2500 pg/ml) <i>n</i> = 235	Trend <i>p</i> -value <sup>a</sup>
<i>Demographic/questionnaire</i>				
Age (years)	70.70	70.97	70.82	0.801
Male, <i>n</i> (%)	143 (59.8)	181 (78.0)	188 (80.0)	<0.001
Northern region, <i>n</i> (%) <sup>b</sup>	103 (43.1)	98 (42.2)	90 (38.3)	0.290
Non-manual occupation, <i>n</i> (%) <sup>b</sup>	114 (50.2)	111 (49.6)	98 (43.8)	0.170
Evidence of CVD, <i>n</i> (%)	40 (16.7)	35 (15.1)	44 (18.7)	0.566
History of diabetes, <i>n</i> (%)	18 (7.5)	25 (10.8)	32 (13.6)	0.033
1–2 alcoholic drinks/day, <i>n</i> (%)	94 (44.8)	105 (48.2)	102 (45.5)	0.882
Current smoker, <i>n</i> (%)	26 (10.9)	32 (13.8)	36 (15.4)	0.150
Physical activity (inactive/occasional), <i>n</i> (%)	131 (58.2)	127 (56.2)	139 (62.1)	0.410
<i>Physical measurements</i>				
Body Mass Index (kg/m <sup>2</sup> ) <sup>c,e</sup>	26.51	26.90	27.69	0.002
Systolic blood pressure (mm/Hg) <sup>c,d,e</sup>	148.02	148.49	149.14	0.621
Diastolic blood pressure (mm/Hg) <sup>c,d,e</sup>	82.62	83.72	83.31	0.530
FEV <sub>1</sub> (L/min) <sup>c,e,g</sup>	2.27	2.23	2.18	0.134
<i>Lipids/metabolic markers</i>				
Total cholesterol (mMol/L)	6.12	6.05	6.07	0.608
HDL cholesterol (mMol/L)	1.52	1.42	1.32	<0.001
Triglyceride (mMol/L) <sup>c,d,f</sup>	1.44	1.57	1.75	<0.001
Insulin (mU/L) <sup>c,d,f</sup>	7.18	8.01	9.36	0.094
Glucose (mMol/L) <sup>c,d,f</sup>	5.76	5.83	5.88	0.235
<i>Inflammatory and hemostatic markers</i>				
C-Reactive Protein (mg/L) <sup>c,f</sup>	1.59	1.74	2.01	0.024
IL-6 (pg/ml) <sup>c,d,f</sup>	2.25	2.39	2.56	0.031
Fibrinogen (g/L) <sup>c,d,f</sup>	3.22	3.22	3.37	0.051
White cell count ( $\times 10^9$ /L) <sup>c,d,f</sup>	6.94	6.78	6.74	0.250
Plasma viscosity (mPa s) <sup>c,d</sup>	1.29	1.28	1.30	0.044
Factor VIII (IU/dL) <sup>c</sup>	142.04	142.18	144.99	0.338
Fibrin D-dimer (ng/ml) <sup>c,f</sup>	80.43	101.78	108.55	<0.001
Von Willebrand factor (IU/dL) <sup>c</sup>	141.45	144.09	155.66	0.001
t-PA (ng/ml) <sup>c,d</sup>	9.83	10.18	10.95	0.002

<sup>a</sup> Sample (*n* = 706) MI controls with IL-18 value. Trend test adjusted for age, gender, region of residence.<sup>b</sup> Reported in 1978–1980 (age 40–59 years) for men and in 1998–2000 (age 60–79 years) for women.<sup>c</sup> Adjusted for age at survey and region of residence (Scotland, North, Midlands and South).<sup>d</sup> Adjusted for time of day.<sup>e</sup> Adjusted for nurse number.<sup>f</sup> Geometric mean, *p* value from linear regression with ln(variable).<sup>g</sup> Adjusted for height squared.

majority to be atherothrombotic with a minority of cardioembolic and lacunar strokes, as atherothrombotic stroke is the commonest type in older adults. IL-18 levels were measured using commercial kits frequently utilized in other epidemiological studies of IL-18 [3]. Whilst cross-sectional and case-control studies cannot exclude reverse causality, the nested case-control design (using prospective data with a mean follow-up time of 6 years for men and 7 years for women) and the sensitivity analyses excluding participants with baseline evidence of either MI or stroke reduce the possibility of the observed associations being due to reverse causality. However, as this is an observational study, we cannot draw firm conclusions about the causal role of IL-18 in development of stroke or CHD. The degree of intra-individual variability in IL-18 levels was measured 4 years apart was estimated using a regression dilution ratio, a considerable advantage over previous prospective studies. The regression dilution ratio of 0.71, suggests similar stability of IL-18 to other established risk factors [15], the variability in IL-18 levels means that estimates of the association between IL-18 and CHD are likely to be conservative estimates of the true association. The sensitivity analyses, testing for differences by gender, age and presence of pre-existing CVD showed similar results to the main analyses but were less precise because of loss of statistical power due to smaller sample size.

#### 4.2. Baseline correlates of IL-18

The distribution of IL-18 observed in the men and women were similar to the age-specific reference ranges reported for the

Glasgow MONICA study, although median levels were slightly lower than in the younger Glasgow study population aged 55–64 years [18]. Whereas the Glasgow study reported lower levels in women, the present study found higher levels in women than in men at a later age (60–79 years).

IL-18 was positively associated with established risk factors blood pressure, HDL-cholesterol and BMI as seen in other studies [18,19]. Not all previous studies [3,19] report associations between IL-18 and total cholesterol and indeed we did not find associations between IL-18 and total cholesterol. IL-18 is reported to be positively associated with adiposity [19] and metabolic syndrome [7], so the associations we observed in controls samples between IL-18 and presence of pre-existing diabetes, BMI, glucose and insulin levels were as expected. The associations seen between IL-18 and other “downstream” inflammatory markers which are emerging risk predictors (fibrinogen, CRP, IL-6, white cell count, plasma viscosity, vWF, Factor VIII, t-PA and D-dimer) were also positive, confirming other reports [18–20].

#### 4.3. IL-18 and risk of CHD

This study did not find consistent evidence that IL-18 was independently associated with risk of MI at conventional levels of statistical significance. Only the age and sex adjusted estimates of the association between continuous IL-18 and MI were statistically significant and associations were abolished on further adjustments, this pattern is similar to three other prospective studies of CHD which included men and women drawn from general populations

**Table 4**Odds ratio (95%CI) of MI in men and women with IL-18 values in the higher tertiles compared to the lower tertile of the distribution ( $n = 895^A$ ).

IL-18 Tertiles	MI cases	MI controls	OR (95%CI) with adjustments		
			Model 1 <sup>b</sup>	Model 2 <sup>c</sup>	Model 3 <sup>c</sup>
Full sample $n = 895$					
Range (pg/mL)					
451–2500	113	199	1.31 (0.92, 1.85)	1.09 (0.75, 1.58)	1.05 (0.72, 1.53)
325–450	100	201	1.15 (0.81, 1.64)	1.13 (0.78, 1.64)	1.13 (0.78, 1.63)
10–324	86	196	1	1	1
Total	299	596	$P = 0.130$	$p = 0.675$	$p = 0.829$
Continuous 1 log <sub>2</sub> (IL-18) <sup>B</sup>	299	596	1.34 (1.04, 1.72)	1.16 (0.88, 1.51)	1.09 (0.83, 1.44)
Sample excluding pre-existing CVD ( $n = 710$ )					
Range (pg/mL)					
451–2500	79	162	1.33 (0.89, 2.00)	1.12 (0.73, 1.73)	1.03 (0.66, 1.61)
325–450	70	170	1.15 (0.76, 1.73)	1.05 (0.68, 1.64)	1.14 (0.74, 1.76)
10–324	62	167	1	1	1
Total	211	499	$P = 0.163$	$p = 0.831$	$p = 0.924$
Continuous 1 log <sub>2</sub> (IL-18) <sup>B</sup>	211	499	1.15 (0.94, 1.41)	1.02 (0.82, 1.27)	1.01 (0.81, 1.26)
Sample with pre-existing CVD ( $n = 185$ )					
Range (pg/mL)					
451–2500	34	37	1.04 (0.49, 2.18)	1.08 (0.49, 2.39)	1.07 (0.48, 2.39)
325–450	30	31	1.08 (0.49, 2.37)	1.15 (0.51, 2.63)	1.14 (0.50, 2.62)
10–324	24	29	1	1	1
Total	88	97	$P = 0.945$	$p = 0.868$	$p = 0.902$
Continuous 1 log <sub>2</sub> (IL-18) <sup>B</sup>	88	97	1.01 (0.70, 1.47)	1.03 (0.70, 1.53)	1.03 (0.69, 1.53)

Model 1 = age, gender and region.

Model 2 = model 1 + SEP + smoking + alcohol + physical activity + history of diabetes + BMI + SBP + DBP + TC + HDL.

Model 3 = model 2 + logIL-6 + logCRP.

<sup>A</sup> Complete case analysis sample. Tertiles based on control group.  $P$  value for test for trend over tertiles.<sup>B</sup> OR of MI per 1 log<sub>2</sub> increase in log<sub>2</sub>(IL-18) i.e. doubling of IL-18.**Table 5**Odds ratio (95%CI) of stroke in men and women with IL-18 values in the higher tertiles compared to the lower tertile of the distribution ( $n = 727^A$ ).

IL-18 Tertiles	Stroke cases	stroke controls	OR (95%CI) with adjustments		
			Model 1 <sup>b</sup>	Model 2 <sup>c</sup>	Model 3 <sup>c</sup>
Full sample $n = 727$					
Range (pg/mL)					
455–2500	83	157	1.24 (0.84, 1.84)	1.07 (0.71, 1.61)	1.09 (0.72, 1.67)
330–454	82	169	1.14 (0.77, 1.68)	1.11 (0.73, 1.69)	1.07 (0.71, 1.61)
10–329	70	166	1	1	1
Total	235	492	$P = 0.287$	$p = 0.626$	$p = 0.685$
Continuous 1 log <sub>2</sub> (IL-18) <sup>B</sup>	299	596	1.26 (0.84, 1.89)	1.13 (0.74, 1.74)	1.11 (0.72, 1.71)
Sample excluding pre-existing CVD ( $n = 594$ )					
Range (pg/mL)					
455–2500	54	132	1.11 (0.71, 1.75)	1.03 (0.64, 1.67)	1.02 (0.63, 1.64)
330–454	62	144	1.17 (0.75, 1.82)	1.09 (0.69, 1.73)	1.10 (0.70, 1.74)
10–329	54	148	1	1	1
Total	170	424	$P = 0.640$	$p = 0.894$	$p = 0.949$
Continuous 1 log <sub>2</sub> (IL-18) <sup>B</sup>	170	424	1.03 (0.74, 1.44)	0.96 (0.67, 1.37)	0.94 (0.66, 1.35)
Sample with pre-existing CVD ( $n = 133$ )					
Range (pg/mL)					
455–2500	29	25	1.94 (0.37, 2.41)	1.94 (0.61, 6.13)	1.97 (0.62, 6.33)
330–454	20	25	1.50 (0.60, 3.72)	1.33 (0.44, 4.04)	1.35 (0.44, 4.16)
10–329	16	18	1	1	1
Total	65	68	$P = 0.331$	$p = 0.246$	$p = 0.239$
Continuous 1 log <sub>2</sub> (IL-18) <sup>B</sup>	65	68	1.42 (0.82, 2.47)	1.73 (0.88, 3.42)	1.74 (0.88, 3.47)

Model 1 = age, gender and region.

Model 2 = model 1 + SEP + smoking + alcohol + physical activity + history of diabetes + BMI + SBP + DBP + TC + HDL.

Model 3 = model 2 + logIL-6 + logCRP.

<sup>A</sup> Complete case analysis sample. Tertiles based on control group.  $P$  value for test for trend over tertiles.<sup>B</sup> OR of MI per 1 log<sub>2</sub> increase in log<sub>2</sub>(IL-18) i.e. doubling of IL-18.

where age and gender adjusted estimates were significant, however further adjustment abolished the associations [19,21,22]. However, the confidence intervals for our estimates of associations between IL-18 and MI overlap with equivalent estimates from the meta-analysis: OR CHD in BRHS/BWHHS 1.31 (95%CI 0.92, 1.85) and OR CHD or CVD in meta-analysis 1.64 (95%CI 1.48, 1.83) [3]. The meta-analysis found statistically significant associations between higher IL-18 and elevated risk of CHD, even after adjustment for age, gender, established risk factors and inflammatory markers IL-6 and CRP [3]. In addition, Mendelian randomization studies in predominantly Caucasian, mostly male populations with average age in mid 50 s or 60 s provide some support for a causal role of IL-18 in risk of CAD or CVD [23,24]. The magnitude of the positive association between continuous IL-18 and MI adjustment for age and sex, in this study of 60–79 year old men and women, was similar to the equivalent adjusted ORs reported in the cohort of men when they were 20 years younger, adjustments for established CVD risk factors and for CRP and IL-6 similarly abolished the associations in both studies [3].

#### 4.4. Associations between IL-18 and stroke

Few epidemiologic studies have investigated how IL-18 is associated with risk of stroke, most evidence comes from studies with composite CVD endpoints including stroke and CHD. The lack of evidence for any elevated risk of stroke associated with IL-18 levels in our study, is consistent with the three previous large studies. In the HOPE study of patients with a history of CAD or stroke or diabetes, IL-18 was not associated with new stroke events during 4.5 years of follow-up [10]. In the PROSPER trial, which recruited patients with vascular risk factors or known vascular disease, there was not evidence that IL-18 was associated with ischemic, hemorrhagic or total stroke over 3.2 years [9]. Similarly in a nested case-control cohort of patients with a history of stroke in the PROGRESS study which included patients with a history of stroke or transient ischemic attack, IL-18 levels were not associated with recurrent ischemic, hemorrhagic or total stroke events over 3.9 years of follow-up [8]. However smaller case-control studies have documented raised IL-18 levels in the post-stroke period [25,26]. In our study we had 80% power to detect a relative risk for stroke of 1.79, assuming two controls per case, so it is possible that our null results may be due to type two error, if the effects are in fact smaller than 1.79, or if the association was diluted due to the mix of both ischemic and hemorrhagic strokes in our study. Future, larger studies including mendelian randomization studies, with data on sub-types of stroke may shed further light on any underlying causal association.

## 5. Conclusions

The results from this study do not support prospective independent associations between IL-18 and risk of MI or stroke in older men and women. The modest association with MI which is attenuated on adjustment for established and novel CHD risk factors, may contribute to understanding the processes underlying risk of MI.

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.cyto.2012.10.010>.

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