**Circulating TNFα levels in older men and women do not show independent prospective relations with MI or stroke**

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**word count body 4642**

**word count abstract 246**

**5 tables, no figures**

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**Abstract**

**Background**

Tumor necrosis factor-alpha (TNFα) is pro-inflammatory cytokine implicated in atherosclerotic plaque formation. We investigated whether circulating TNFα is prospectively associated with myocardial infarction (MI) or stroke in the older general population, independently of established cardiovascular risk factors and other inflammatory markers related to CHD risk.

**Methods**

We measured baseline TNFα concentrations in stored serum samples of 362 incident MI and 299 incident stroke cases and controls (2 per case, frequency matched by age, gender and town) who were 'nested' in parallel prospective studies of 4252 men and 4286 women aged 60-79 years assessed in general practices in 24 British towns in 1998-2000 and followed-up for an average 7 years for fatal and non-fatal MI and stroke.

**Results**

TNFα levels were 11.4% (95% CI 9.5, 13.3%) higher among MI cases than controls; geometric mean 1.84 pg/mL compared to 1.63pg/mL, p(difference)<0.001. Participants in the top third of baseline TNFα levels had an age-adjusted odds ratio (OR) for MI of 1.75 (95%CI 1.22, 2.49) compared with those in the bottom third, which was reduced to 1.47 (95%CI 1.01, 2.14) after adjustment for established cardiovascular risk factors. However, further adjustment for C-reactive protein and interleukin-6 abolished the association OR 1.33 (95% CI 0.91, 1.66) and the linear trend. Excluding subjects with pre-existing CVD did not materially affect results. No significant association between TNFα and stroke was observed.

**Conclusions**

This study suggests that TNFα is not a strong independent risk marker for MI, and is not associated with risk of stroke.

**Key words** Myocardial infarction, stroke, inflammation, epidemiology, TNFα, prospective,

cohort                                                                                               3

**Abbreviations**

TNFα Tumor necrosis factor-alpha

MI myocardial infarction

CVD cardiovascular disease

CHD coronary heart disease

OR Odds Ratio

IQR inter-quartile range

CI confidence interval

CRP C-reactive protein

IL-6 interleukin-6

BMI body mass index

SBP systolic blood pressure

DBP diastolic blood pressure

t-PA tissue plasminogen activator

LR Likelihood Ratio

Introduction

Inflammation is important in the development and rupture of atherosclerotic lesions leading to cardiovascular disease (CVD) events(1). Population-based prospective studies report associations between circulating acute phase inflammatory markers and CHD (coronary heart disease)(2;3) and stroke(4). Evidence is accumulating that pro-inflammatory cytokines (eg interleukin 6 [IL-6]) are related to CHD risk(5). Tumor necrosis factor alpha (TNFα) is a pro-inflammatory cytokine important in initiating inflammatory responses(6). Human studies and animal models implicate TNFα in atherosclerotic plaque formation (7;8). TNFα is produced by macrophages, foam cells and mast cells (among others), promotes cellular infiltration of the plaque and stimulates production of other cytokines which increase plaque instability leading to thrombus formation(9).

Higher circulating levels of TNFα might be expected to be associated with increased CHD and stroke risks based on experimental evidence, but epidemiologic evidence is inconsistent. Some(10-12) but not all(13) cross-sectional data indicate positive associations between TNFα and degree of atherosclerosis or level of prevalent CVD, and some studies of secondary CHD report null findings (13;14). There are few prospective studies of healthy individuals(11;12), and they include fewer than 300 cases of hard CHD endpoints (11-13;15). Some studies report positive associations between TNFα and primary CHD risk (11;12). Even fewer studies of TNFα and stroke exist, the largest (n=591 cases of recurrent stroke) reported positive associations between TNFα and stroke(16), although findings for primary stroke risk have been null(11;17). Considerable uncertainty remains about the strength of association of TNFα with risk of MI and stroke in generally healthy population cohorts.

We investigate associations between serum TNFα and risk of MI and stroke in two large parallel prospective population-based studies of older adult men and women, and examine

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whether associations are independent of established cardiovascular risk factors and inflammatory markers implicated in cardiovascular risk.

Methods

In 1998-2000, 4252 men from one General Practice in each of 24 British towns participating in a prospective study of CVD were followed-up at age 60-79 years (response rate 77%)(18). In 1999-2001, a parallel study of 4286 women of the same age and in the same Practices was established, with the addition of one town (Bristol)(19). Near identical protocols for the collection of data were used in both studies. Nurses made physical measurements, recorded an electrocardiogram and collected fasting venous blood samples. Serum was stored at $\leq$-70$^{o}$C for subsequent analysis. Participants were followed-up for all cause mortality and cardiovascular morbidity, for between 6.25 and 8.5 years with a loss of <2%. Fatal cases were ascertained through National Health Service Central Registers using death certificates (ICD-9 codes 410-414) for MI and (ICD-9 codes 430-438) for stroke, indicating deaths with cerebrovascular disease as the underlying cause. Diagnosis of non-fatal MI or stroke was based on reports from General Practitioners and reviews of General Practice records, and was in accordance with World Health Organisation criteria(18;20). Participants completed detailed questionnaires about previous illnesses including MI or stroke, and lifestyle including cigarette smoking, alcohol consumption and physical activity. Own longest-held occupation (or husband's occupation for married, divorced and widowed women) was coded using the Registrar General's classification. Town of residence was recorded for women in 1998-2000 and for men at the start of their follow-up in 1978-80. Weight, height and seated blood pressure were measured and fasting blood samples provided for the measurement of cholesterol, triglycerides and haemostatic and inflammatory markers (5;18;19;21). All subjects provided written informed consent to the investigation and ethical approval was provided by relevant local research ethics committees.

We established a nested case-control study using all 390 MI cases occurring between examination (1998-2000) and June 2006 in men and between examination (1999-2001) and September 2007 in women. A total of 780 controls "frequency matched" to cases on town of residence, gender and age in 5-year bands were randomly selected from survivors free from incident CHD at the end of follow-up. Similarly a separate nested case-control study of 324 cases of stroke and 648 controls was established. Numbers of MI and stroke cases were constrained by the numbers of relevant events, so two controls per case were used to increase statistical power and precision of estimates of relative risks between TNF$\alpha$, MI and stroke as well as with other CVD risk factors. In the sample with complete data, there were 362 MI cases, from which we would have had 80% power to detect a relative risk of 1.59 in the top tertile of TNF$\alpha$ compared to the bottom tertile, at the 5% statistical significance level. From 299 cases of stroke we would have had 80% power to detect a relative risk of 1.68 in the top tertile of TNF$\alpha$ compared to the bottom tertile at the 5% statistical significance level, assuming 2 controls per case.

TNF$\alpha$ (pg/mL) was measured from stored frozen serum samples using a commercially available high-sensitivity ELISA (R&D systems, Abingdon, UK) by an investigator blinded to case-control status of the samples. The coefficient of variation was 8.4% intra-assay and 12.5% inter-assay. In 158 men from two towns, TNF$\alpha$ was measured in samples obtained both in 1996 and in 2000 to enable intra-individual comparisons over time (regression dilution) (22).

*Statistical methods*

Highly skewed variables were natural log transformed. 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 TNFα were defined in the MI control sample. Associations between TNFα tertiles and covariates were examined using one way ANOVA. Unmatched logistic regression analyses were used to examine associations between TNFα tertiles and MI. Models were first adjusted for gender, age and region. Further adjustments for cardiovascular risk factors (selected *a priori*) were fitted as continuous variables except for; smoking (current, ex or never), alcohol use (1-2 drinks/day or other) physical activity (more or less than 3 hours of moderate/ vigorous activity per week), and history of diabetes (present or absent).

For MI, regression models were first adjusted for glucose and insulin (both natural log transformed) and then for novel risk factors which are consistently associated with CHD (log transformed C reactive protein (CRP) and IL-6). For stroke, adjustments were made for CRP and IL-6. Linear regression was used to model the association between risk of MI (or stroke) and continuous TNFα both as linear and $\log_2$ transformation (to assess the effect of a doubling of TNFα level). Gender and age differences in the TNFα-CVD associations were tested using likelihood ratio (LR) tests for interactions. All reported p values are 2-sided. Sensitivity analyses excluded participants with pre-existing CVD (self-report of MI or stroke at any questionnaire between 1978-80 and 1998-2000 in men and in 1999/2000 questionnaire in women).

The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written

Results

*Baseline Characteristics*

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For the MI case-control groups, data were available for 362 of 390 cases (267 men and 95 women, mean age 70.9 years) and for 698 of 780 controls. Cases differed from controls in having higher prevalence of pre-existing CVD and diabetes (Table 1). Cases had higher prevalence of smokers, fewer drinkers of 1-2 units of alcohol/day and fewer physically active participants (all $p<0.05$). They had higher systolic blood pressure (SBP) and lower forced expiratory volume ($FEV_1$) but similar body mass index (BMI) and diastolic blood pressure (DBP). Cases had less favorable lipid and inflammatory markers. Geometric mean $TNF\alpha$ level was 11.4% (95% CI 9.5, 13.3%) higher in MI cases than controls; 1.84 pg/mL (IQR 1.29, 2.42) compared to 1.63pg/mL (IQR 1.19, 2.18) p(difference) <0.001.

$TNF\alpha$ data were available for 299 of 324 stroke cases and 587 of 648 controls. Mean age of cases was 71.3 years, and 63% were male. Cases had higher prevalence of pre-existing CVD, diabetes and smokers than controls (all $p<0.05$). They had higher blood pressure and lower $FEV_1$ but similar BMI. Cases had lower total cholesterol and higher IL-6, fibrinogen and white blood cell count (although not CRP). Differences between cases and controls were less marked than in the MI study population. $TNF\alpha$ levels were slightly higher among the stroke cases than controls 1.88pg/mL (IQR 1.28, 2.34) compared to 1.75 pg/mL (IQR 1.32, 2.36) p=0.079 (Table 2).

*Baseline correlates of TNF$\alpha$*

Table 3 presents associations between $TNF\alpha$ and potential confounders across thirds of the MI control population (the larger control population). Increasing $TNF\alpha$ levels were associated with female gender, but not with age or behavioral factors. Increasing $TNF\alpha$ was associated with higher BMI, triglyceride, insulin and glucose (borderline) and lower HDL. $TNF\alpha$ was also positively associated with CRP, IL-6, fibrinogen, white blood cell count, plasma viscosity, coagulation factor VIII, D-dimer, von Willebrand factor (vWF) and tissue-plasminogen activator (t-PA). Not all correlations between natural log $TNF\alpha$ and covariates

reached levels of conventional statistical significance (p<0.05), those that did reach significance were all of moderate magnitude r<0.25 (e.g. r=0.17, (p<0.05) for CRP and r=0.16, (p<0.05) for IL-6; r=0.20 (p<0.05) for plasma viscosity). Similar associations were observed in the stroke control population (data not shown).

*Association of TNFα with risk of MI*

Table 4 summarizes associations between MI and tertiles of TNFα. Model 1 was adjusted for gender, age and region of residence; the odds ratio (OR) for MI associated with the highest compared to the lowest TNFα tertile was 1.75 (95%CI 1.22, 2.49) and there was a significant linear trend in ORs across the tertiles (p=0.002). After adjustment for established, behavioral and metabolic cardiovascular risk factors, the OR was attenuated to 1.47 (95%CI 1.01, 2.14) but the linear trend remained (Table 4). Adjustment for CRP and IL-6 attenuated the OR; 1.33 (95% CI 0.91, 1.96) and abolished the linear trend. A doubling of TNFα level was associated with increased OR for MI: 1.34 (95%CI 1.11, 1.60), which was reduced on adjustment for established and metabolic risk factors, and completely attenuated by adjustment for inflammatory markers. There was no evidence that associations between MI and tertiles of TNFα varied by gender or age (LR test p=0.101, p=0.972 respectively). Likewise, interactions were not observed for $\log_2$ TNFα and gender or age (LR test p=0.684, p=0.720 respectively).

Repeating analyses excluding 180 participants with pre-existing CVD, produced similar results, although point estimates were wider due to loss of power and estimates were further attenuated by adjustments, eliminating the trend in CHD.

*Association of TNFα with risk of stroke*

No significant associations between TNFα and stroke were observed in multivariate models (Table 5). OR for stroke associated with top compared to bottom TNFα tertile was 1.12

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(95%CI 0.75, 1.66). There was no evidence of an interaction between gender and TNFα on stroke (LR test p=0.319), or age (LR test p=1). There was not evidence of an association between continuous TNFα and stroke; OR for doubling of TNFα 1.15 (95%CI 0.95, 1.39). Excluding 133 participants with prior CVD confirmed main analysis results.

*Extent of regression dilution effect*

Using Rosner's method (23), a regression dilution ratio of 0.25 (95%CI 0.15, 0.35) for TNFα was calculated in 158 men with TNFα levels measured 4 years apart (22).

Discussion

In this, the largest prospective population-based study reported to date on serum TNFα levels and risk of CVD, higher serum TNFα levels were associated with elevated MI risk in older adults over a 7-year period. However, the association was partly attenuated by established and metabolic risk factors, and reduced to non-significance by adjustment for circulating inflammatory markers associated with CHD: CRP (2;4) and IL-6 (5). However, TNFα was not associated with risk of stroke.

*Comparison with previous studies*

TNFα was positively associated with some established risk factors: BMI and inversely with HDL as reported in a cross-sectional study (24), but associations were not observed with total cholesterol or blood pressure as seen in some but not all other studies (10;24). TNFα is reported to be an adipokine and is associated with metabolic processes (25;26), so positive associations between TNFα and BMI, glucose and insulin observed here were expected. Associations between TNFα and other "downstream" inflammatory markers which are emerging risk predictors (fibrinogen, CRP, white cell count, plasma viscosity, vWF, Factor VIII, t-PA and D-dimer) were modestly positive, similar to other reports(11;24) and unlikely

to result in collinearity. Higher TNFα among females was unexpected, but may be because women in this study were post-menopausal(24).

The magnitude of the positive association of TNFα with MI adjusted for established risk factors fits with other findings (11;12). To deal with the different cut-points for TNFα tertiles between studies, the association between continuous TNFα and MI risk was examined. The adjusted OR for $\log_{10}$ TNFα in our study (1.18 (95%CI 1.00, 1.39) was very similar to association reported elsewhere: RR 1.22 (1.04, 1.43) and their RR for stroke was also null, like our study(12). The Finnish study (of younger adults) reported non-linearity in the elevated risks of MI and total mortality: men in the three highest quartiles of TNFα (>0.75 pg/L) had similarly raised HR for MI 2.21 (95%CI 1.18-4.14) compared to the lowest quartile (12). However in our study, the highest TNFα tertile (>1.89 pg/mL) had the most elevated odds ratio and linear trends were observed across the tertiles. Finally, the lack of association between TNFα and stroke is consistent with a recent study in the elderly at risk(17). Our study confirms this finding in a generally healthier population of older people.

*Strengths and weaknesses*

This is the largest study of TNFα, CHD and stroke; it includes more events than previous prospective population-based studies, has a clinically relevant follow-up, and includes both genders. The data add to the literature because few other studies report prospective data about TNFα and subsequent risk of either MI (11;12;27;28) or stroke (11) in generally healthy populations. The study population is representative of gender, social background and geographical variation across the UK. This study benefits from validated data about MI and stroke events collected from GP practices using standardized procedures and then individually verified for accuracy. The validity of this procedure has been previously reported(29). The study also has comprehensive data on established and novel cardiovascular risk factors. TNFα levels were measured blind to case status, using standard laboratory

protocols on well-preserved samples. The distributions of TNFα were similar to age-specific reference ranges (24). Patterns of association between TNFα and cardiovascular risk factors were similar to other studies Although a single measure of TNFα per subject was available, measurement of intra-individual variability over 4 years in a subsample of 158 men is a strength (22).

*Interpretation of results*

This study suggests that associations between TNFα and MI are modest, although the high intra-individual variability in TNFα levels measured 4 years apart, suggests lower long-term biological stability of TNFα (in terms of expression) than other risk factors(22). The low regression dilution ratio (0.25) is similar to that of another pro-inflammatory cytokine IL-6, suggesting that, like IL-6, the association of TNFα with incident CHD may be underestimated in studies with single measurements(5). The modest associations observed between TNFα and MI are unlikely to be only due to the wide range of established biological, social or behavioural risk factors we measured (since these were adjusted for in regression models),,or due to participants having pre-existing CVD: sensitivity analyses excluded such participants.

Associations between TNFα and MI were independent of insulin and glucose, fitting with data from a prospective study of diabetic women, where CHD risks were elevated independent of hyperglycemia (27). Associations between TNFα and MI were attenuated particularly when CRP and IL-6 (separately or together) were included in multivariate analyses, whilst CRP and IL-6 remained independently associated with MI, suggesting that each could be important pathways from TNFα to MI risk. Most studies of TNFα and MI have not adjusted for other inflammatory markers. One study reported that adjustment for CRP, partially attenuated the association between TNFα and CHD but abolished the association with total mortality, however that study did not include IL-6(11). Adjusting for any inflammatory markers may be over-adjustment in terms of aetiology, since TNFα is thought

to act synergistically with IL-6 and IL-1, upstream of the acute phase response(30). However in our study, no statistical interaction between TNFα and IL-6 on MI or stroke was observed. Hence in biological terms, the association of "downstream" acute-phase reactants (such as CRP, fibrinogen or white cell count) with CVD endpoints could be primarily due to increased levels of circulating TNFα, IL-6 and potentially other pro-inflammatory cytokines (5;7;8). The attenuation of associations of TNFα with outcome confirm findings in a recurrent stroke study, that a profile of raised inflammatory markers (rather than any specific independent inflammatory marker) is associated with vascular risk(16). Hence TNFα does not add much to other inflammatory markers in predicting CHD risk. For example, CRP which probably has more robust associations with CHD risk, may not much improve risk prediction(4). Overall, due to relatively modest associations with CHD risk, a relatively high degree of regression dilution, and confounding by generalized inflammation, TNFα is unlikely to be a clinically useful risk biomarker, although this does not diminish a potential role in CHD aetiology.

*Conclusions*

Our study provides further evidence that TNFα is associated with a modestly elevated risk of MI, after adjustment for established cardiovascular risk factors, although this association is not independent from inflammatory markers CRP or IL-6. Associations with stroke were not observed. The modest association with MI may give insights into the process of MI risk, but TNFα is unlikely to be a clinically useful risk marker for identifying patients at risk of CHD.

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**Table 1. Baseline Characteristics (age 60-79) of the MI case and control populations: mean (SD) unless otherwise specified[*].**

| | MI Cases (n=362) | MI Controls (n=698) | Difference (p value) |
|---|---|---|---|
| **Demographic/questionnaire** | | | |
| Age (years) | 70.91 (5.48) | 70.80 (5.44) | Matched |
| Male, n(%) | 267 (73.6) | 512 (73.1) | Matched |
| Northern region of residence, n(%)[†] | 150 (41.0) | 286 (41.4) | Matched |
| Non-manual occupation, n(%)[†] | 152 (44.4) | 319 (47.9) | 0.298 |
| Prior evidence of MI or stroke, n(%) | 107 (29.6) | 117 (16.8) | <0.001 |
| History of diabetes, n(%) | 55 (20.6) | 55 (10.7) | <0.001 |
| 1-2 alcoholic drinks/day, n(%) | 126 (37.8) | 299 (46.4) | 0.010 |
| Current smoker, n(%) | 75 (20.72) | 93 (13.3) | 0.004 |
| Physical activity (inactive/occasional), n(%) | 231 (66.4) | 391 (58.6) | 0.016 |
| | | | |
| **Physical measurements** | | | |
| Body mass index (Kg/m$^2$)[‡‖] | 27.11 (4.26) | 27.02 (3.95) | 0.728 |
| Systolic blood pressure (mmHg)[‡§‖] | 153.94 (27.22) | 148.55 (24.16) | 0.001 |
| Diastolic blood pressure (mmHg)[‡§‖] | 83.85 (12.07) | 83.22 (11.52) | 0.409 |
| FEV$_1$ (L/min)[‡‖#] | 2.15 (0.55) | 2.23 (0.60) | 0.028 |
| | | | |
| **Lipids / Metabolic markers** | | | |
| Total cholesterol (mMol/L) | 6.24 (1.22) | 6.08 (1.07) | 0.027 |
| HDL cholesterol (mMol/L) | 1.31 (0.33) | 1.41 (0.36) | <0.001 |
| Triglyceride (mMol/L)[‡§¶] | 1.80 (1.32, 2.40) | 1.58 (1.14, 2.12) | <0.001 |

| | | | |
|---|---|---|---|
| Insulin (mU/L)$^{\ddagger\S\P}$ | 9.25 (5.57, 13.61) | 7.85 (5.37, 11.07) | <0.001 |
| Glucose (mMol/L)$^{\ddagger\S\P}$ | 6.19 (5.36, 6.38) | 5.84 (5.37, 6.12) | <0.001 |

**Inflammatory and hemostatic markers**

| | | | |
|---|---|---|---|
| TNFα(pg/ml)$^{\ddagger\P}$ | 1.84 (1.29, 2.42) | 1.63 (1.19, 2.18) | <0.001 |
| C-Reactive Protein (mg/L)$^{\dagger\P}$ | 2.41(1.08, 5.58) | 1.77 (0.90, 3.60) | <0.001 |
| IL-6(pg/ml)$^{\ddagger\S\P}$ | 2.99 (1.89, 4.26) | 2.43 (1.54, 3.47) | <0.001 |
| Fibrinogen(g/L)$^{\ddagger\S\P}$ | 3.47 (3.03, 3.98) | 3.26 (2.85, 3.75) | <0.001 |
| White cell count (^$10^9$/L)$^{\ddagger\S\P}$ | 7.27 (6.01, 8.84) | 6.80 (5.68, 7.98) | <0.001 |

[*]Case-control sample is maximum available

[†] reported in 1978-1980(age 40-59 years) for men and in 1998-2000 (age 60-79 years) for women

[‡] adjusted for gender, age at survey and region of residence (Scotland, North, Midlands and South).

[§] adjusted for time of day

[‖] adjusted for nurse number

[¶]analysed as natural log transformed variable, geometric mean (IQR) reported on original scale.

[#] adjusted for height squared

**Table 2. Baseline Characteristics (age 60-79) of the stroke case and control populations: mean (SD) unless otherwise specified[*].**

| | Stroke Cases (n=299) | Stroke Controls (n=587) | Difference (p value) |
|---|---|---|---|
| **Demographic/questionnaire** | | | |
| Age (years) | 71.29 (5.32) | 71.35 (5.24) | Matched |
| Male, n(%) | 191 (63.4) | 372 (63.9) | Matched |
| Northern region, n(%)[†] | 108 (36.1) | 219 (37.3) | Matched |
| Non-manual occupation, n(%)[†] | 148 (54.8) | 290 (52.9) | 0.609 |
| Prior evidence of MI or stroke, n(%) | 84 (28.1) | 89 (15.2) | <0.001 |
| History of diabetes, n(%) | 31 (16.2) | 37 (10.0) | 0.030 |
| 1-2 alcoholic drinks/day, n(%) | 105 (38.6) | 210 (38.5) | 0.937 |
| Current smoker, n(%) | 51 (17.1) | 57 (9.7) | 0.004 |
| Physical activity (inactive/occasional), n(%) | 196 (71.0) | 377 (66.8) | 0.223 |
| | | | |
| **Physical measurements** | | | |
| Body mass index $(Kg/m^2)$ [‡‖] | 27.05 (4.37) | 27.12 (4.11) | 0.814 |
| Systolic blood pressure (mmHg) [‡§‖] | 154.10 (25.01) | 149.13 (24.24) | 0.005 |
| Diastolic blood pressure (mmHg) [‡§‖] | 84.84 (12.60) | 82.36 (11.18) | 0.003 |
| $FEV_1$ (L/min) [‡‖#] | 2.09 (0.57) | 2.24 (0.52) | <0.001 |
| | | | |
| **Lipids / Metabolic markers** | | | |
| Total cholesterol (mMol/L) | 6.12 (1.22) | 6.31 (1.17) | 0.024 |
| HDL cholesterol (mMol/L) | 1.43 (0.40) | 1.42 (0.36) | 0.736 |
| Triglyceride (mMol/L) [‡§¶] | 1.64 (1.10, 2.31) | 1.69 (1.23, 2.29) | 0.363 |

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| | | | |
|---|---|---|---|
| Insulin (mU/L) ‡§¶ | 8.53 (5.31, 12.02) | 7.96 (5.03, 10.93) | 0.159 |
| Glucose (mMol/L) ‡§¶ | 6.05 (5.34, 6.19) | 5.88 (5.32, 6.12) | 0.085 |

**Inflammatory and hemostatic markers**

| | | | |
|---|---|---|---|
| TNF$\alpha$ (pg/ml)‡¶ | 1.88 (1.28, 2.34) | 1.75 (1.32, 2.36) | 0.079 |
| C-Reactive Protein (mg/L)†¶ | 2.12 (1.03, 4.44) | 1.92 (0.91, 4.16) | 0.239 |
| IL-6(pg/ml)‡§¶ | 2.83 (1.85, 4.01) | 2.56 (1.64, 3.53) | 0.026 |
| Fibrinogen(g/L)‡§¶ | 3.35 (2.95, 3.84) | 3.28 (2.87, 3.75) | 0.142 |
| White cell count (^$10^9$/L)‡§¶ | 7.22 (6.14, 8.56) | 6.90 (5.74, 8.20) | <0.001 |

[*] case control sample is maximum available

[†] reported in 1978-1980(age 40-59 years) for men and in 1998-2000 (age 60-79 years) for women

[‡] adjusted for gender, age at survey and region of residence (Scotland, North, Midlands and South).

[§] adjusted for time of day

[‖] adjusted for nurse number

[¶]analysed as natural log transformed variable, geometric mean (IQR) reported on original scale.

[#] adjusted for height squared

**Table 3. Association between TNFα (tertiles) and cardiovascular risk factors in the MI control sample (n=698)**

| | Low (0.03-1.3) N= 233 | Medium (1.31-1.88) N= 234 | High (1.89-13.56) n= 231 | Trend p-value[*] |
|---|---|---|---|---|
| **Demographic/questionnaire** | | | | |
| Age (years) | 70.30 | 70.70 | 71.40 | 0.090 |
| Male, n(%) | 171 (73.4) | 184 (78.6) | 157 (68.0) | 0.034 |
| Northern region, n(%)[†] | 96 (41.2) | 94 (40.2) | 96 (41.6) | 0.951 |
| Non-manual occupation, n(%)[†] | 113 (51.6) | 106 (47.5) | 100 (44.6) | 0.339 |
| Evidence of stroke, n(%) | 20 (11.7) | 17 (9.2) | 25 (15.9) | 0.165 |
| History of diabetes, n(%) | 16 (9.4) | 21 (11.4) | 18(11.5) | 0.773 |
| 1-2 alcoholic drinks/day, n(%) | 102 (48.3) | 107 (48.2) | 90 (42.7) | 0.407 |
| Current smoker, n(%) | 33 (14.2) | 23 (9.9) | 37 (16.0) | 0.131 |
| Physical activity (inactive/occasional), n(%) | 125 (56.3) | 126 (55.5) | 140 (64.2) | 0.122 |
| **Physical measurements** | | | | |
| Body mass index $(Kg/m^2)$ [‡‖] | 26.38 | 27.42 | 27.24 | 0.001 |
| Systolic blood pressure (mmHg) [‡§‖] | 148.59 | 149.33 | 147.66 | 0.757 |
| Diastolic blood pressure (mmHg) [‡§‖] | 83.04 | 82.81 | 83.81 | 0.065 |
| $FEV_1$ (L/min) [‡‖#] | 2.27 | 2.23 | 2.17 | 0.240 |
| **Lipids / Metabolic markers** | | | | |
| Total cholesterol (mMol/L) | 6.09 | 6.09 | 6.06 | 0.914 |
| HDL cholesterol (mMol/L) | 1.50 | 1.43 | 1.32 | <0.001 |
| Triglyceride (mMol/L) [‡§¶] | 1.44 | 1.59 | 1.71 | <0.001 |
| Insulin (mU/L) [‡§¶] | 7.28 | 7.64 | 8.67 | 0.004 |
| Glucose (mMol/L) [‡§¶] | 5.71 | 5.88 | 5.94 | 0.056 |

**Inflammatory and hemostatic markers**

| | | | | |
|---|---|---|---|---|
| C-Reactive Protein (mg/L)[‡¶] | 1.41 | 1.74 | 2.25 | <0.001 |
| IL-6(pg/ml)[‡§¶] | 2.20 | 2.33 | 2.80 | <0.001 |
| Fibrinogen(g/L)[‡§¶] | 3.16 | 3.26 | 3.37 | 0.012 |
| White cell count (^10$^9$/L)[‡§¶] | 6.61 | 6.64 | 7.14 | 0.003 |
| Plasma viscosity (mPa.s)[‡§] | 1.27 | 1.29 | 1.31 | <0.001 |
| Factor VIII (IU/dL)[‡] | 141.35 | 138.52 | 148.09 | 0.005 |
| Fibrin D-dimer (ng/ml)[‡¶] | 80.74 | 91.18 | 118.99 | <0.001 |
| Von Willebrand Factor (IU/dL)[‡] | 139.61 | 142.16 | 157.31 | <0.001 |
| t-PA (ng/ml)[‡§] | 9.74 | 10.32 | 11.14 | <0.001 |

[*]sample (n=698) MI controls with TNFα value. Trend test adjusted for age, gender, region of residence

[†] reported in 1978-1980(age 40-59 years) for men and in 1998-2000 (age 60-79 years) for women

[‡] adjusted for age at survey and region of residence (Scotland, North, Midlands and South).

[§] adjusted for time of day

[‖] adjusted for nurse number

[¶] geometric mean, p value from ANOVA with ln(variable)

[#] adjusted for height squared

**Table 4. Odds Ratio (95% CI) of MI in men and women with TNFα values in the higher tertiles compared to the lower tertile of the distribution (n=867)[*]**

| TNFα | MI cases | MI controls | OR (95% CI) with adjustments | | | |
|---|---|---|---|---|---|---|
| Tertiles | | | Model 1 | Model 2 | Model 3 | Model 4 |
| Range (pg/mL) | | | | | | |
| 1.89-22.90 | 128 | 186 | 1.75 (1.22, 2.49) | 1.47 (1.01, 2.14) | 1.44 (0.99, 2.11) | 1.33 (0.91, 1.96) |
| 1.31-1.88 | 84 | 203 | 1.03 (0.71, 1.48) | 0.99 (0.67, 1.44) | 0.98 (0.67, 1.44) | 0.94 (0.64, 1.39) |
| 0.03-1.30 | 77 | 189 | 1 | 1 | 1 | 1 |
| Total | 289 | 578 | P=0.002 | p=0.038 | P=0.049 | P=0.125 |
| | | | | | | |
| Continuous | | | Model 1 | Model 2 | Model 3 | Model 4 |
| 1 $\log_2$ (TNFα)[†] | 289 | 578 | 1.34 (1.11, 1.60) | 1.25 (1.03, 1.51) | 1.23 (1.02, 1.49) | 1.18 (0.97, 1.43) |

[*] complete case analysis sample. Tertiles based on control group. P value for test for trend over tertiles

[†] OR of MI per 1 $\log_2$ increase in $\log_2$(TNFα) ie doubling of TNFα

Model 1= age, gender and region

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

Model 3= model 2 + insulin+ glucose

Model 4 =model 3 + IL-6 +CRP

**Table 5. Odds Ratio (95% CI) of stroke in men and women with TNFα values in the higher tertiles compared to the lower tertile of the distribution (n=722)[*]**

| TNFα Tertiles Range (pg/mL) | Stroke cases | Stroke controls | OR ( 5 % CI) with adjustments | | |
|---|---|---|---|---|---|
| | | | Model 1 | Model 2 | Model 3 |
| 2.07-45.30 | 78 | 161 | 1.12 (0.75, 1.66) | 1.15 (0.76, 1.74) | 1.11 (0.73, 1.69) |
| 1.40-2.06 | 80 | 155 | 1.19 (0.81, 1.75) | 1.13 (0.75, 1.69) | 1.11 (0.74, 1.66) |
| 0.03-1.39 | 76 | 172 | 1 | 1 | 1 |
| Total | 234 | 488 | p=0.567 | p=0.516 | p=0.631 |
| | | | OR (95% CI) | | |
| Continuous | | | Model 1 | Model 2 | Model 3 |
| 1 $\log_2$ (TNFα)[†] | 234 | 488 | 1.15 (0.95, 1.39) | 1.14 (0.93, 1.40) | 1.13 (0.92, 1.38) |

[*] complete case analysis sample. Tertiles based on control group. P value for test for trend over tertiles

[†] OR of MI per 1 $\log_2$ increase in $\log_2$(TNFα) ie doubling of TNFα

Model 1= age, gender and region

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

Model 3= model 2 + IL-6 + CRP