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# Relationship of serum C3 to fasting insulin, risk factors and previous ischaemic events in middle-aged men

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**Aims** Serum C3 is a powerful indicator of the risk of myocardial infarction, which correlates with body mass index, serum lipids and blood pressure. This study was performed to ascertain whether such correlations may be explained by an association of C3 with fasting insulin, and to assess comparatively the relationships of C3 and traditional risk factors to previous myocardial infarction.

**Methods and Results** The fasting levels of C3, insulin, and the main risk factors were evaluated in 1090 unselected men aged 55-64 years, including 129 cases of previous ischaemic events (51 myocardial infarctions). In multivariate analysis C3 was associated with insulin (r=0·27, $P<0·0001$), cholesterol (r=0·18, $P<0·0001$), body mass index (r=0·13, $P<0·0001$), glucose (r=0·12, $P=0·0001$), systolic blood pressure (r=0·10, $P<0·001$), triglycerides (r=0·09, $P<0·01$) and HDL-cholesterol (r= − 0·06, $P<0·05$). These variables explained 31% of the total C3 variance. Alcohol consumption and physical activity correlated inversely with C3, while no correlation was found with smoking and family history of myocardial infarction. C3 was associated with previous myocardial infarction and stroke, but not with angina pectoris and peripheral arterial disease. In logistic regression the variables associated with previous myocardial infarction were C3 ($P=0·011$), family history of myocardial infarction ($P=0·018$), ex-smoker status ($P=0·020$), age ($P=0·025$), glucose ($P=0·028$) and HDL-cholesterol ($P=0·051$, inverse relationship).

**Conclusions** The association of C3 with myocardial infarction persists retrospectively, and is more significant than any other association of traditional risk factors with previous myocardial infarction. Of the many variables associated with C3, fasting insulin is its main covariate, which suggests that C3 is a marker of a pro-atherogenic metabolic imbalance partly coinciding with insulin resistance.

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**Key Words:** Complement-3, insulin, risk factors, myocardial infarction.

**See page 1036 for the Editorial comment on this article**

## Introduction

C3, the third complement component, is a cytokine produced by activated macrophages[1], which are the cells mostly implicated in the formation of 'vulnerable' atherosclerotic plaques[2]. Moreover, C3 is an acute phase reactant produced by the liver[3] in response to interleukin-1 which, in turn, is secreted by the activated macrophages in the inflammation sites[4].

Serum C3 is a powerful indicator of the risk of myocardial infarction in men: the risk at 4 years for subjects with C3 levels in the high tertile was found to be tenfold with respect to the remaining population[5]. This relationship seems to be specific for myocardial infarction, since no prospective association was demonstrated with angina pectoris and the manifestations of extra-coronary atherosclerosis.

In addition, an increasing amount of data shows that serum C3 correlates with several metabolic risk factors. We found a strong correlation with body mass

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index[6], which probably reflects C3 synthesis by adipocytes[7]. Other independent correlations were found with low density lipoprotein and total cholesterol[6,8,9], triglycerides[6,8–10], blood glucose[6] and systolic blood pressure[6,11]. All or some of these risk factors often coexist in the presence of insulin resistance[12].

Fasting insulin has been used in large population studies as an index of insulin resistance[13,14] and has been found to be an additional and independent risk factor compared to more traditional risk factors for myocardial infarction[15]. Thus, with the present study we tried to ascertain whether a relationship between serum C3 and fasting insulin exists, and whether such a relationship explains the correlation between C3 and other risk factors. Moreover, we had the opportunity to verify all the above correlations in a wide homogeneous unselected sample, and to assess other relationships previously not considered.

## Methods

### *Subjects*

The San Vitale Study is designed to ascertain the spontaneous variability of C3 and the feasibility of changing its serum concentrations by dietary or pharmacological interventions. The data reported here were collected during the initial screening survey, between 19 May 1997 and 22 July 1997. By a letter illustrating the study characteristics, 1840 men aged 55–64 years, resident in the San Vitale district of Bologna, were invited in alphabetical order (names and addresses were provided by the local registry office). The relationship between age and C3 had been studied in a previous survey[6], in which a much wider age range was considered. The age range 55-64 was chosen to obtain information on men at a pre-senile age with a maximum probability of developing ischaemic events, which will be useful as a design for a follow-up study. Non-responders were sent a second letter. Of the 1111 (60·4%) participants 21 were excluded because of missing data. Thus, the cohort examined in this study consisted of 1090 unselected men (mean age 58·8 ± 2·9 (1 SD) years).

Through a standardized questionnaire, each subject reported his height, as well as family history of myocardial infarction (first-degree relative affected before 60 years of age), previous history of hypertension, diabetes or ischaemic diseases, physical activity (at least 90 min per week of heavy work or sport), smoking habit and alcohol consumption (number of 'drinks' per week: one pint of beer, one glass of wine or one small glass of spirit were considered a drink, i.e. approximately 8 g of alcohol). The subjects with a history of hypertension or systolic blood pressure $\geqq$160 mmHg or diastolic blood pressure $\geqq$95 mmHg were considered hypertensives. Subjects with a history of diabetes or blood glucose levels $\geqq$7·8 mmol . $l^{-1}$ (140 mg . $dl^{-1}$) were considered diabetics.

All subjects were seen twice: the first time, in the morning, they provided informed written consent and a fasting blood sample. The second time, after a week and in the afternoon, they provided information on acute (during the last month) or chronic diseases and the possible use of drugs, they proffered documentation on previous ischaemic diseases and possible interventions of arterial revascularization (including bypass, thromboendarterectomy and percutaneous angioplasty procedures); finally, their weight and blood pressure were measured. Each of the ischaemic events was considered in the subsequent analysis only if confirmed by a hospital record, a written diagnosis, or an unequivocal instrumental investigation. The prescribed medications were: ACE-inhibitors (13·4%), calcium antagonists (10·0%), diuretics (9·6%), beta-blockers (9·1%), antiplatelet drugs (8·8%), oral antidiabetic drugs (4·9%), lipid lowering drugs (4·8%), non-steroidal antiinflammatory drugs (1·4%) and corticosteroids (0·8%). Of the 108 diabetics, 32 (30%) were untreated, 22 (20%) were treated with diet alone and 54 (50%) were prescribed drugs: sulfonylureas and biguanides (n=27; 25%), sulfonylureas alone (n=20; 18%), biguanides alone (n=5; 5%) or sulfonylureas and insulin (n=2; 2%). The insulin and glucose levels of the two insulin treated subjects were 47·4 and 67·2 pmol . $l^{-1}$, and 11·7 and 16·0 mmol . $l^{-1}$, respectively. The study protocol was approved by the joint university–hospital Ethical Committee.

### *Measurements*

Blood pressure was measured by a mercury sphygmomanometer to the nearest mmHg, with the subject sitting up, at the end of the visit. Body mass index was obtained dividing body weight by the square of height (kg . $m^{-2}$). Venous blood samples were allowed to clot at room temperature and then centrifuged. An aliquot of fresh serum was used to measure, on the same day of sampling, C3, total cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides and glucose. C3 was measured by nephelometry[16] (Behring kits, Behringwerke AG, Marburg, Germany) according to the International Federation of Clinical Chemistry (IFCC) standard. The coefficient of variation was <4·4% for measurements on the same day, and <5·5% for measurements performed on different days. Serum lipids were measured by enzymatic methods[17,18] (Boehringer Mannheim GmBH, Mannheim, Germany). Their coefficients of variation were, respectively, for within a day and day-to-day measurements, <2% and <3% for total cholesterol, <3·5% and <4% for HDL cholesterol, and <2% and <3% for triglycerides. Insulin (immunoreactive insulin) was measured with a two-site immunoenzymometric assay (AIA-PACK IRI Tosoh, Tokyo, Japan, no cross reactivity with C peptide) with the sera frozen at − 80 °C (the coefficient of variation both within a day and day-to-day was <8%). Immunoenzymatic methods for insulin measurement generally compared favourably with radioimmunoassay in sensitivity and precision[19].

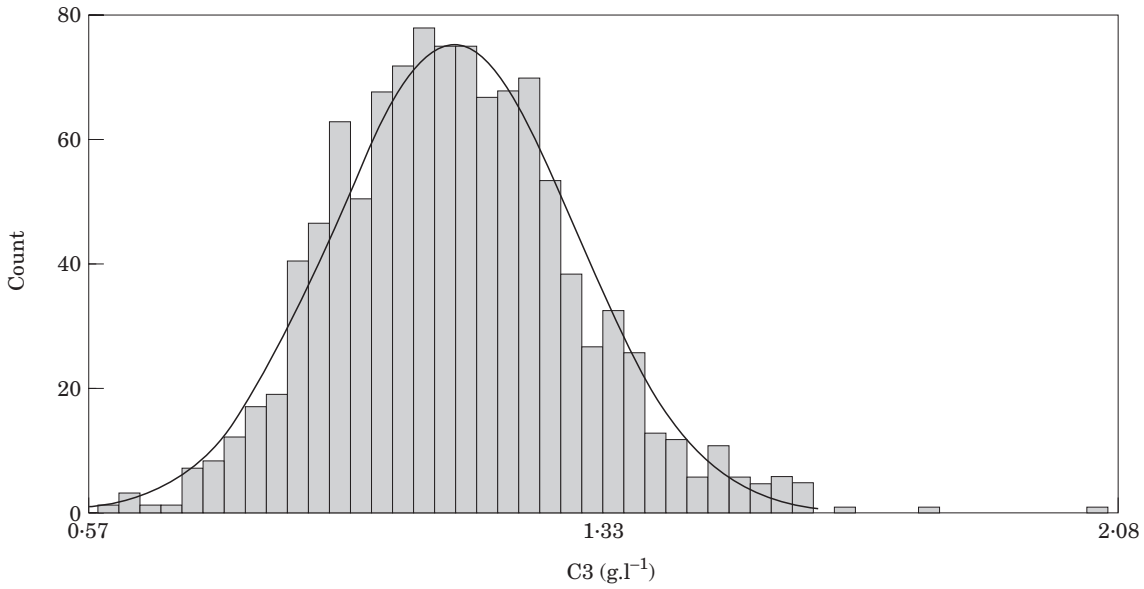


***Figure 1*** **Distribution of serum C3 levels in 1090 unselected men aged 55–64 years. After removal of the three highest outliers the distribution is substantially normal: skewness 0·25, kurtosis 0·04, $P<0·05$ for normality test.**

The lower limit of detection of the assay was 12 pmol . $l^{-1}$ (2 μU . $ml^{-1}$). Values equal or possibly lower than 12 pmol . $l^{-1}$ were found in 13 subjects (1·2%).

### *Statistical analysis*

Since the distribution of blood glucose, insulin and triglycerides was not normal (marked positive skewness), univariate analysis was performed with non-parametric tests: Mann–Whitney's test for comparisons between two groups, Kruskal–Wallis ANOVA for comparisons among three or more groups, and Spearman's test to assess simple correlations between two variables. The chi-square test was used to compare percentages. Multivariate analysis was performed by multiple linear regression, when both dependent and independent variables were continuous. For this analysis, the three variables with skewed distribution were log-transformed. When the dependent variable was of a dichotomous type, multiple logistic regression models were used. Both types of multiple regression (linear and logistic) were repeated several times, each time excluding from the model the variable with the least significant association to the dependent variable: the analysis was concluded when all the remaining variables were significantly associated with the dependent variable ('backward' elimination procedure). The statistical analysis was performed with SOLO-BMDP Statistical Software (Version 4.0, BMDP, Los Angeles, U.S.A.). All deviations from the mean were expressed as 1 SD. Two-tail tests were used throughout. The significance level was set at $P=0·05$.

## Results

### *Relationship between C3 and risk factors*

C3 distribution in the study population was substantially normal (Fig. 1). Values ranged from 0·59 to 2·06 g . $l^{-1}$, with mean and standard deviations of 1·11 and 0·18 g . $l^{-1}$, respectively. The two tertile limits were 1·02 and 1·19 g . $l^{-1}$.

Table 1 shows the mean levels of C3 in relation to the clinical characteristics obtained by interview or questionnaire. No relationship was found with age, family history of myocardial infarction and chronic bronchitis (cough and expectoration for more than 3 months/year in the last 2 years). In decreasing order of significance, hypertension, diabetes and a recent acute inflammation (especially influenza) were associated with higher C3 levels.

Past smokers had significantly higher C3 levels than current smokers and those who had never smoked. Among past smokers, with respect to the other two groups considered together, there was a greater prevalence of previous interventions of arterial revascularization (6·3 vs 1·3%; $P<0·0001$), previous myocardial infarction (7·4 vs 3·2%; $P<0·0001$), previous ischaemic events in general (16·0 vs 9·6%; $P<0·002$) and diabetes (13·1 vs 7·3%; $P<0·002$). All these conditions are associated with increased C3 levels (see further) and may

***Table 1 C3 levels according to clinical history***

| Variable | Status | Number (%) | Serum C3 ($g \cdot l^{-1}$) | *P* value |
|---|---|---|---|---|
| Age (years) | <59 | 525 (48·2) | 1·10 ± 0·18 | 0·1144 |
| | ≧59 | 565 (51·8) | 1·11 ± 0·17 | |
| FH-MI | No | 926 (85·0) | 1·11 ± 0·18 | 0·6406 |
| | Yes | 164 (15·0) | 1·11 ± 0·18 | |
| Physical activity | No | 713 (65·4) | 1·13 ± 0·18 | <0·0001 |
| | Yes | 377 (34·6) | 1·07 ± 0·16 | |
| Alcohol (drinks/week) | <3 | 363 (33·3) | 1·13 ± 0·17 | 0·0001 |
| | ≧3, <14 | 345 (31·6) | 1·11 ± 0·17 | |
| | ≧14 | 382 (35·1) | 1·08 ± 0·18 | |
| Smoker | Never | 374 (34·3) | 1·09 ± 0·17 | 0·0007 |
| | Past | 380 (34·9) | 1·14 ± 0·18 | |
| | Current | 336 (30·8) | 1·09 ± 0·18 | |
| Hypertension | No | 664 (60·9) | 1·08 ± 0·17 | <0·0001 |
| | Yes | 426 (39·1) | 1·16 ± 0·18 | |
| Diabetes | No | 982 (90·1) | 1·10 ± 0·17 | <0·0001 |
| | Yes | 108 (9·9) | 1·20 ± 0·17 | |
| Acute inflammation | No | 923 (84·7) | 1·10 ± 0·18 | 0·0003 |
| (<1 month) | Yes | 167 (15·3) | 1·15 ± 0·17 | |
| Chronic bronchitis | No | 989 (90·7) | 1·11 ± 0·17 | 0·5836 |
| | Yes | 101 (9·3) | 1·12 ± 0·19 | |

C3 levels are expressed as mean ± 1 SD.
FH-MI = Family history of myocardial infarction.

induce patients to stop smoking. Moreover, past smokers had higher body mass index values (27·7 ± 3·9 vs 26·2 ± 3·7 $kg \cdot m^{-2}$; $P<0·0001$), insulin (64·8 ± 72·0 vs 51·6 ± 31·2 $pmol \cdot l^{-1}$; $P<0·0001$) and blood glucose (6·08 ± 2·11 vs 5·58 ± 1·44 $mmol \cdot l^{-1}$; $P<0·0001$). When all of these variables were included in a multiple logistic regression model with the past smoker status as the dependent variable, only body mass index ($P<0·0001$), previous arterial revascularization ($P=0·0001$) and blood glucose ($P<0·05$), but not C3, remained associated with the past-smoker status. Thus, there is no independent association between C3 and cigarette smoking.

By a similar analysis, alcohol consumption over the median (>7 drinks/week, n=512) was found to be independently associated with HDL cholesterol ($P<0·0001$), diabetes ($P=0·007$, inverse relation), total cholesterol ($P=0·006$), as well as C3 ($P<0·02$, inverse relation). Similarly, physical activity was independently associated with C3 ($P=0·0001$, inverse relation), HDL cholesterol ($P=0·008$, direct relation) and a recent acute inflammation ($P=0·02$, inverse relation).

All the continuous variables measured in this study were very significantly associated with C3 ($P \leqq 0·0001$, Table 2). The strongest associations were with insulin (see also Fig. 2), triglycerides and body mass index.

## *Univariate and multivariate correlations among variables*

Table 3 gives the complete matrix of simple non-parametric correlations among the continuous variables, including alcohol consumption (drinks/week) and cigarette smoking (cigarettes/day). The strongest correlations (rho >0·3), in decreasing order of rho coefficient, were between systolic and diastolic blood pressure, body mass index and insulin, triglycerides and HDL cholesterol (inverse relation), C3 and insulin, C3 and triglycerides, cholesterol and triglycerides and, finally, insulin and triglycerides. The correlation between C3 and insulin was also strong among the 108 diabetics (rho=0·37, $P=0·0001$).

Multiple linear regression models were tested to ascertain which of the relationships found were independent. Each variable was considered dependent on the variables associated with it with rho coefficients >0·1. Alcohol consumption and cigarette smoking were not included in this analysis. Table 4 reports the partial r coefficients and significance levels of all the significant relationships found, which are graphically summarized in Fig. 3. The variable involved in the greatest number of independent correlations (n=7) was C3, followed by HDL cholesterol (n=6), body mass index (n=5), insulin, blood glucose and triglycerides (n=4), total cholesterol and systolic blood pressure (n=3) and diastolic blood pressure (n=2).

C3 was independently associated with all the variables assessed, except diastolic blood pressure. The strongest association was, again, with fasting insulin, followed by the association with total cholesterol. When insulin was not included among the independent variables, the other six associations with C3 remained significant, but the overall $R^2$ decreased (from 0·31 to 0·26) and the partial r coefficients for the relationships with body mass index and triglycerides increased (respectively, from 0·13 to 0·27 and from 0·09 to 0·14).

***Table 2 C3 levels according to tertiles of measured variables***

| Variable | Mean ± SD [range] | Tertile limits | Number | Serum C3 ($g \cdot l^{-1}$) | *P* value | T |
|---|---|---|---|---|---|---|
| BMI ($kg \cdot m^{-2}$) | 26·8 ± 3·8 | <26 | 424 | 1·05 ± 0·16 | <0·0001 | 98·9 |
| | [13–44] | ≧26, <28 | 283 | 1·11 ± 0·16 | | |
| | | ≧28 | 383 | 1·17 ± 0·18 | | |
| SBP (mmHg) | 136·1 ± 20·4 | <126 | 388 | 1·07 ± 0·17 | 0·0001 | 29·8 |
| | [90–220] | ≧126, <143 | 337 | 1·11 ± 0·18 | | |
| | | ≧143 | 365 | 1·14 ± 0·17 | | |
| DBP (mmHg) | 80·7 ± 10·7 | <76 | 372 | 1·08 ± 0·17 | 0·0001 | 19·2 |
| | [50–130] | ≧76, <85 | 301 | 1·11 ± 0·18 | | |
| | | ≧85 | 417 | 1·14 ± 0·18 | | |
| Glucose ($mmol \cdot l^{-1}$) | 5·77 ± 1·72 | <5·11 | 352 | 1·06 ± 0·17 | <0·0001 | 47·2 |
| | [2·44–20·10] | ≧5·11, <5·66 | 360 | 1·11 ± 0·17 | | |
| | | ≧5·66 | 378 | 1·15 ± 0·18 | | |
| TC ($mmol \cdot l^{-1}$) | 5·69 ± 0·98 | <5·27 | 363 | 1·07 ± 0·17 | <0·0001 | 46·8 |
| | [2·81–9·48] | ≧5·27, <6·10 | 362 | 1·10 ± 0·17 | | |
| | | ≧6·10 | 365 | 1·15 ± 0·17 | | |
| TG ($mmol \cdot l^{-1}$) | 1·75 ± 1·13 | <1·18 | 364 | 1·04 ± 0·16 | <0·0001 | 115·2 |
| | [0·39–9·77] | ≧1·18, <1·82 | 363 | 1·11 ± 0·16 | | |
| | | ≧1·82 | 363 | 1·18 ± 0·17 | | |
| HDL-C ($mmol \cdot l^{-1}$) | 1·44 ± 0·29 | <1·32 | 376 | 1·15 ± 0·18 | <0·0001 | 58·4 |
| | [0·67–2·32] | ≧1·32, <1·60 | 370 | 1·11 ± 0·18 | | |
| | | ≧1·60 | 344 | 1·06 ± 0·16 | | |
| Insulin ($pmol \cdot l^{-1}$) | 56·4 ± 49·8 | <36 | 346 | 1·01 ± 0·14 | <0·0001 | 208·6 |
| | [12·0–1233·6] | ≧36, <60 | 381 | 1·11 ± 0·16 | | |
| | | ≧60 | 363 | 1·20 ± 0·17 | | |

C3 levels are expressed as mean ± 1 SD.
T values have been obtained by Kruskal–Wallis ANOVA.
BMI=body mass index, DBP=diastolic blood pressure, HDL-C=high density lipoprotein cholesterol, SBP=systolic blood pressure, TC=total cholesterol, TG=triglycerides.

The strongest correlation involving insulin was with body mass index, followed by the correlations with C3, triglycerides and HDL cholesterol. When C3 was removed from the model in which insulin was the dependent variable, then insulin was also associated with diastolic blood pressure (partial r=0·067; $P$=0·028) and blood glucose (partial r=0·066; $P$=0·030).

Blood glucose was independently associated with C3 and body mass index with similar partial r coefficients (0·15 and 0·14, respectively). Among the diabetic subjects, only these two associations were present. In the overall population, blood glucose was also associated with systolic blood pressure and, inversely, with HDL cholesterol. Only when C3 was removed from the model, were triglycerides (partial r=0·12; $P$<0·0001) and insulin (partial r=0·06; $P$<0·05) also associated with blood glucose.

Systolic blood pressure correlated strongly with diastolic blood pressure and, more weakly, with blood glucose and C3. The univariate relationship between systolic blood pressure and insulin was not confirmed, since it was due to the common association of these two variables with C3 (Fig. 3).

## *Relationship between C3 and previous ischaemic events*

Table 5 shows that mean C3 levels were increased in the subjects with previous ischaemic events and procedures of arterial revascularization. Among ischaemic events, the association with C3 mainly concerned previous myocardial infarction and, to a lower extent, previous stroke. C3 was not associated with angina pectoris (in the subjects who had not had a myocardial infarction), peripheral arterial disease and transient cerebral ischaemic attacks.

Among the variables studied, an increased prevalence of previous myocardial infarction (Table 6) was associated, in decreasing order of significance, with C3, HDL cholesterol (inverse association), blood glucose, triglycerides and insulin (continuous variables), and diabetes, past-smoker status, family history of myocardial infarction and hypertension (dichotomous variables).

To ascertain which associations with previous myocardial infarction were independent, the nine variables above and age were included in a multiple logistic regression model. Significant independent associations were found with C3 levels ($P$=0·011), family history of myocardial infarction ($P$=0·018), past-smoker status ($P$=0·020), age ($P$=0·025), blood glucose ($P$=0·028) and HDL cholesterol ($P$=0·051, inverse relation). Glucose level proved to be stronger than the definition of diabetes. Insulin, triglycerides and hypertension were not independently associated with previous myocardial infarction. Results were substantially the same when the model did not include C3, except for an increase in significance for the associations

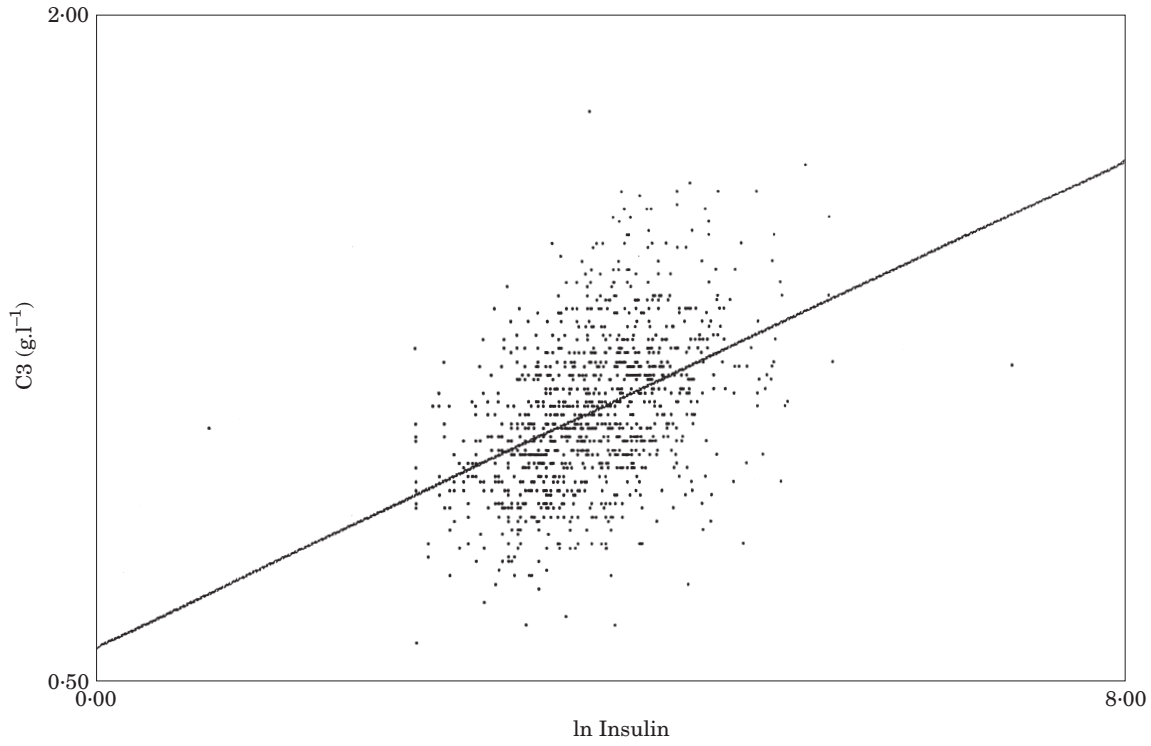


***Figure 2*** **Simple linear regression between the natural logarithm of fasting insulin [ln (pmol . l$^{-1}$)] and C3 levels. Equation of regression line is: Y=0·138 X+0·576. Pearson's r=0·442. *P*<0·0001.**

***Table 3*** ***Univariate correlation matrix of risk factors***

| | Alcohol | Smoke | BMI | SBP | DBP | Glucose | TC | TG | HDL-C | Insulin | C3 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Alcohol | — | **0·15** | − 0·06 | − 0·01 | 0·01 | 0·03 | **0·13** | 0·02 | **0·21** | − 0·09 | **− 0·14** |
| Smoke | | — | **− 0·12** | − 0·06 | − 0·07 | **− 0·12** | 0·02 | **0·11** | **− 0·12** | **− 0·14** | − 0·08 |
| BMI | | | — | **0·24** | **0·29** | **0·23** | − 0·00 | **0·22** | **− 0·24** | **0·56** | **0·35** |
| SBP | | | | — | **0·67** | **0·17** | 0·08 | 0·09 | − 0·01 | **0·18** | **0·20** |
| DBP | | | | | — | 0·06 | 0·06 | 0·09 | 0·04 | **0·20** | **0·15** |
| Glucose | | | | | | — | 0·05 | **0·15** | **− 0·13** | **0·26** | **0·23** |
| TC | | | | | | | — | **0·34** | **0·13** | 0·01 | **0·22** |
| TG | | | | | | | | — | **− 0·53** | **0·33** | **0·35** |
| HDL-C | | | | | | | | | — | **− 0·28** | **− 0·23** |
| Insulin | | | | | | | | | | — | **0·46** |

Continuous variables only. Alcohol consumption measured as number of drinks per week. Smoking measured as number of cigarettes per day. Numbers are Spearman's rho coefficients. Coefficients ≧0·1 ($P$<0·001) are marked. The correlations are significant ($P$<0·05) for rho coefficients >0·06.
BMI=body mass index, DBP=diastolic blood pressure, HDL-C=high density lipoprotein cholesterol, SBP=systolic blood pressure, TC=total cholesterol, TG=triglycerides.

with blood glucose ($P$=0·004) and HDL cholesterol ($P$=0·010).

## Discussion

Similar to what has been found prospectively[5], in a large population of middle-aged men, serum C3 was the variable most significantly associated with previous myocardial infarction in multivariate analysis. Furthermore, C3 was the variable correlated with the greatest number of risk factors. This may simply reflect the fact that risk factors induce atherosclerosis, and activated macrophages in atherosclerotic plaques produce C3[1]. However, this study has also shown that, among risk factors, insulin is the one most strongly associated with C3, which suggests that the correlations of C3 with some risk factors may be mediated, at least in part, by insulin resistance. Other acute phase proteins, such as PAI-1 , fibrinogen and C-reactive protein, also correlate with insulin[13,20,21]. There are at least two explanations for these correlations. First, both the synthesis of acute

***Table 4 Significant multivariate independent associations among risk factors***

| Dependent variable | BMI | SBP | DBP | Glucose | TC | TG | HDL-C | Insulin | C3 | $R^2$ | F |
|---|---|---|---|---|---|---|---|---|---|---|---|
| BMI | — | | 0·21‡ | 0·14‡ | | | − 0·10‡ | 0·41‡ | 0·11† | 0·36 | 123·3 |
| SBP | | — | 0·67‡ | 0·17‡ | | | | | 0·08* | 0·49 | 344·1 |
| DBP | 0·17‡ | 0·66‡ | — | | | | | | | 0·48 | 502·2 |
| Glucose | 0·14‡ | 0·11† | | — | | | − 0·10† | | 0·15‡ | 0·11 | 35·2 |
| TC | | | | | — | 0·48‡ | 0·43‡ | | 0·15‡ | 0·30 | 157·6 |
| TG | | | | | 0·49‡ | — | − 0·57‡ | 0·16‡ | 0·10† | 0·50 | 267·8 |
| HDL-C | − 0·09* | | | | 0·42‡ | − 0·58‡ | — | | − 0·07 | 0·41 | 190·6 |
| Insulin | 0·44‡ | | | | | 0·11† | − 0·07 | — | 0·26‡ | 0·38 | 169·7 |
| C3 | 0·13‡ | 0·10† | | 0·12‡ | 0·18‡ | 0·09* | − 0·06 | 0·27‡ | — | 0·31 | 70·2 |

Multiple linear regression; continuous variables only, with normal or normalized distribution; tested models included, as independent variables, only those variables associated with their dependent variable with a Spearman's rho coefficient $\geqq 0{\cdot}1$ in univariate analysis. Numbers are partial r coefficients.
BMI=body mass index; DBP=diastolic blood pressure; HDL-C=high density lipoprotein cholesterol; SBP=systolic blood pressure; TC=total cholesterol; TG=triglycerides.
*$P<0{\cdot}01$, †$P<0{\cdot}001$, ‡$P<0{\cdot}0001$ (no symbol for $P<0{\cdot}05$).

phase proteins in the liver and insulin resistance are induced by cytokines such as tumour necrosis factor[22]. As far as the source of such cytokines is concerned, Yudkin *et al.* have recently proposed that this may be the adipose tissue, rather than some chronic infection[23]. Second, insulin is known to inhibit the hepatic synthesis of acute phase proteins, including C3[24]. Thus, insulin resistance, besides causing an increase in insulin levels, would also reduce this inhibition, with a subsequent increase in acute phase protein production.

In multivariate analysis, however, the relationship between risk factors and C3 persisted after the addition

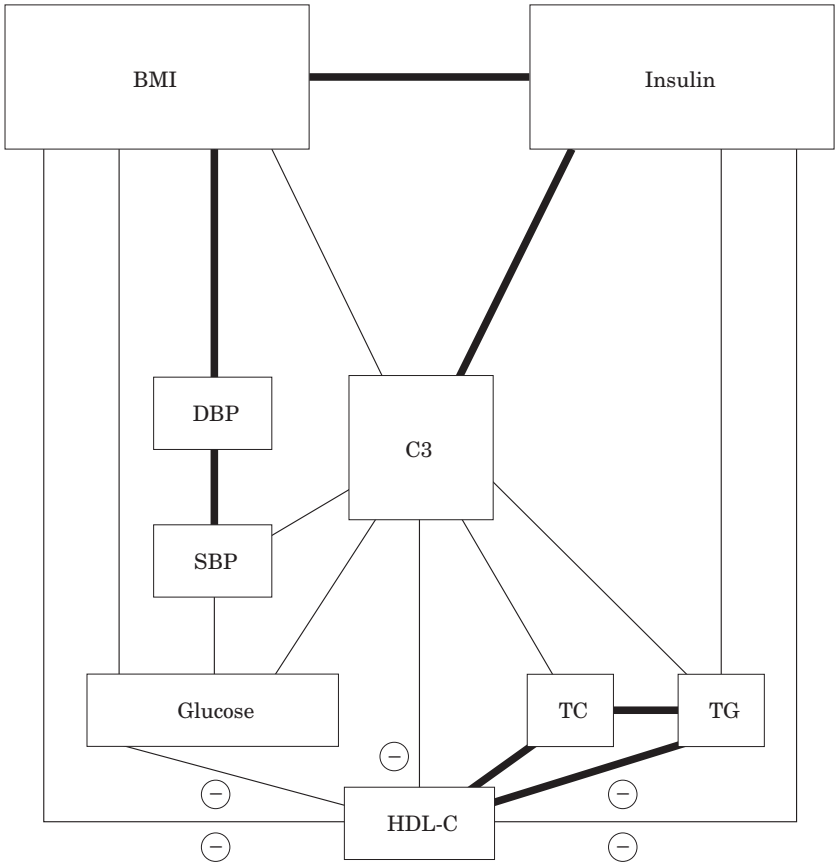


***Figure 3* Schematic representation of significant independent relationships among continuous variables, ascertained by multiple linear regression models. Bold lines indicate partial r coefficients $\geqq 0{\cdot}2$. Minus signs indicate inverse relationships.**

***Table 5 C3 levels according to previous events***

| Previous event | Status | Number | Serum C3 ($g \cdot l^{-1}$) | *P* value |
|---|---|---|---|---|
| Myocardial infarction | No | 1039 | 1·10 ± 0·18 | <0·0001 |
| | Yes | 51 | 1·21 ± 0·17 | |
| Angina pectoris (without MI) | No | 1060 | 1·11 ± 0·18 | 0·2396 |
| | Yes | 30 | 1·15 ± 0·19 | |
| Stroke | No | 1074 | 1·11 ± 0·18 | 0·0051 |
| | Yes | 16 | 1·24 ± 0·18 | |
| TIA | No | 1082 | 1·11 ± 0·18 | 0·5502 |
| | Yes | 8 | 1·07 ± 0·16 | |
| Peripheral arterial disease | No | 1058 | 1·11 ± 0·18 | 0·0857 |
| | Yes | 32 | 1·16 ± 0·19 | |
| Arterial revascularization | No | 1057 | 1·11 ± 0·18 | 0·0022 |
| | Yes | 33 | 1·20 ± 0·17 | |
| Any of the above | No | 961 | 1·10 ± 0·18 | <0·0001 |
| | Yes | 129 | 1·17 ± 0·17 | |

Serum C3 levels are expressed as mean ± 1 SD.
TIA=transient cerebral ischemic attack.

***Table 6 Prevalence of previous myocardial infarction according to exposure levels of risk factors***

| Risk factor | Low tertile | Middle tertile | High tertile | $\chi^2$ | *P* value |
|---|---|---|---|---|---|
| BMI | 14 (3·3) | 16 (5·6) | 21 (5·5) | 2·96 | 0·2275 |
| SBP | 21 (5·4) | 14 (4·1) | 16 (4·4) | 0·75 | 0·6882 |
| DBP | 18 (4·8) | 19 (6·3) | 14 (3·4) | 3·39 | 0·1835 |
| Glucose | 12 (3·4) | 11 (3·1) | 28 (7·4) | 9·70 | 0·0078 |
| TC | 21 (5·8) | 15 (4·1) | 15 (4·1) | 1·49 | 0·4738 |
| TG | 9 (2·5) | 16 (4·4) | 26 (7·2) | 9·05 | 0·0108 |
| HDL-C | 25 (6·6) | 21 (5·7) | 5 (1·4) | 12·09 | 0·0024 |
| Insulin | 12 (3·5) | 14 (3·7) | 25 (6·9) | 5·89 | 0·0526 |
| C3 | 9 (2·4) | 14 (3·9) | 28 (7·7) | 12·36 | 0·0021 |
| | No | Yes | | | |
| Diabetes | 37 (3·4) | 14 (13·0) | | 18·45 | <0·0001 |
| Hypertension | 23 (3·5) | 28 (6·6) | | 5·62 | 0·0177 |
| FH-MI | 37 (4·0) | 14 (8·5) | | 6·44 | 0·0111 |
| Current smoker | 39 (5·2) | 12 (3·6) | | 1·34 | 0·2478 |
| Past smoker | 23 (3·2) | 28 (7·4) | | 9·46 | 0·0021 |
| Alcohol >7 dr./week | 27 (4·7) | 24 (4·7) | | 0·00 | 0·9899 |

Numbers are count and per cent prevalence (in parentheses). See Table 2 for definition of variable tertiles. BMI=body mass index; DBP=diastolic blood pressure; FH-MI=family history of myocardial infarction; HDL-C=high density lipoprotein cholesterol; SBP=systolic blood pressure; TC=total cholesterol; TG=triglycerides.

of insulin to the independent variables. Moreover, serum C3 and body mass index, but not insulin, were the main covariates of blood glucose. Finally, some known relationships between insulin and risk factors (namely, blood pressure and blood glucose) were confirmed in univariate analysis, but disappeared after inclusion of C3 in a multivariate model in which insulin was the dependent variable. Thus, serum C3 might be a better marker of insulin resistance than insulin itself. This possibility could be tested by more direct measures of insulin resistance, such as the euglycaemic hyperinsulinaemic clamp, which unfortunately are not applicable to large population studies. It seems probable, however, that some of the relationships between C3 and risk factors are not entirely explained by insulin resistance, being also sustained by other mechanisms. One of these may be the above mentioned connection among risk factors, atherosclerosis, activated macrophages and C3, which more suitably explains the strong relationship with cholesterol, and is in agreement with the recently reported association between atherosclerotic plaques with a 'soft' echostructure and C3 levels[25]. Furthermore, C3a-des-Arg, a fragment of C3 coinciding with acylation-stimulating protein[26], is the most potent agent stimulating triglyceride synthesis and glucose membrane transport in human adipocytes[27]. Finally, the 'ring' of correlations among systolic blood pressure, glucose and C3 (Fig. 3) suggests the possible

involvement of corticosteroid hormones which, in fact, are known to induce the production of acute phase proteins in the liver[28] and whose incretion is promoted by cytokines through pituitary stimulation[29].

Alcohol consumption is independently associated with lower C3 levels, which may be a consequence of the alcohol induced inhibition of macrophage activation and inflammatory cytokine production[30].

The inverse relationship between physical activity and C3 is very significant and independent of the relationship to other variables. Exercise causes complement activation with following C3 consumption and, possibly, inhibition of C3 synthesis[31]. Also, catecholamines are known to inhibit the production of inflammatory cytokines[32].

In a recent study we found that smokers had lower C3 levels than non-smokers[6]. The present investigation has shown that past smokers, who had previously been classified within non-smokers, have higher C3 levels than both never and current smokers. The occurrence of some ischaemic event, or the presence of glucose intolerance, are often good reasons to decide to stop smoking. Moreover, body mass index tends to increase after stopping smoking. When all these confounders (which per se may cause an increase in C3 levels) were accounted for, no independent relationship between smoking and C3 was demonstrable.

The absolute lack of an association between C3 and a family history of myocardial infarction suggests that the association between C3 and myocardial infarction is not related to genetic causes. Serum C3 was also associated with previous stroke, but not with previous angina pectoris, peripheral arterial disease or transient ischaemic attack, which substantially confirms our prospective results[5]. This might have been because stable angina pectoris and peripheral arterial disease are more frequently associated with fibrous stable plaques with relatively low lipid and inflammatory components, while acute coronary syndromes are often due to the presence of lipid and macrophage-rich, 'vulnerable', plaques[2]. This interpretation would also agree with the lack of an association between cigarette smoking and C3: smoking is typically associated with fibrous arteriosclerotic lesions in the lower limbs, while it would be mainly involved in acute coronary syndromes as a precipitating agent[33].

## Conclusions

The main findings of this study, concerning a large unselected population of middle-aged men, show that: (1) the independent association between serum C3 and myocardial infarction persists after the acute event and, indeed, is more significant than any other association between risk factors and previous myocardial infarction; (2) compared to other variables, serum C3 is associated with the greatest number of traditional risk factors; (3) the main covariate of C3 is fasting insulin; (4) the main covariates of blood glucose are C3 and body mass index. Although an inflammation occurring in vulnerable coronary plaques or elsewhere may contribute to C3 elevation, these results strongly suggest that the link between C3 and myocardial infarction is mainly represented by a pro-atherogenic metabolic imbalance coinciding, at least in part, with insulin resistance.

This interpretation might also apply to other acute phase reactants, which are known to correlate with both risk factors[34,35] and fasting insulin[13,20,21]. However, C3 may play a specific role in atherogenesis, since its fragment C3a-des-Arg exerts important functions in the control of lipid and glucose metabolism[26,27]. Due to these properties, and the strict prospective and retrospective association with myocardial infarction, serum C3 deserves consideration as one of the most promising markers for the assessment of the risk of acute coronary events.

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