Complement C3 Is a Risk Factor for the Development of Diabetes

A Population-Based Cohort Study

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Cross-sectional studies have reported strong correlations between plasma levels of complement C3, insulin, and glucose. This prospective study explored whether elevated levels of C3, C4, and other inflammation-sensitive plasma proteins (ISPs; fibrinogen, orosomucoid, α1-antitrypsin, haptoglobin, and ceruloplasmin) are associated with the development of diabetes. Plasma proteins were measured in 2,815 nondiabetic healthy men, age 38–50 years, who were reexamined after a mean follow-up of 6.1 years. Diabetes development (n = 123) was studied in relation to baseline levels of plasma proteins. After adjusting for age, screening year, and glucose at baseline, the odds ratio (95% CI) for developing diabetes was 1.00, 2.4 (1.1–5.3), 2.9 (1.4–6.0), and 5.6 (2.8–10.9), respectively, for men with C3 in the 1st, 2nd, 3rd, and 4th quartiles (trend: $P < 0.00001$). Fibrinogen, haptoglobin, C4, and the number of elevated ISPs were also related to future diabetes in this model. Only C3 was significantly associated with diabetes development after further adjustments for potential confounders, including BMI, insulin, and other inflammatory markers. We concluded that the risk of developing diabetes is related to levels of complement C3. *Diabetes* 54:570–575, 2005

Complement C3 and C4 are the major plasma proteins of the immune system complement pathways. The synthesis of these proteins is increased in response to inflammation and infection but at a slower rate than for traditional acute phase proteins (1,2). Both C3 and C4 have shown substantial correlations with obesity (3–6), and high gene expression of these complement components has been reported in omental adipose tissue in obese men (3). High C3 levels have been reported in subjects with diabetes and insulin resistance (6–9).

It has been shown that the cleavage product of C3, acylation-stimulating protein (ASP), is a paracrine metabolic factor that stimulates the uptake of glucose and fat storage in human adipose tissue (10–12). ASP deficiency in mice has been associated with resistance to weight gain on a high-fat diet, despite increased food intake (13). However, whether C3 is associated with an increased risk of developing diabetes is unknown.

Several inflammatory markers have been associated with the incidence of diabetes, including C-reactive protein (CRP) (14–17), orosomucoid (18), sialic acid (18), white blood cells (18,19), and interleukin (IL)-6 (20). It has been proposed that diabetes is a disease of the innate immune system (21). However, several studies have reported a nonsignificant relation between inflammation and incidence of diabetes. Negative or inconclusive results have been reported for CRP (17,22), haptoglobin (18), fibrinogen (16,18), white blood cells (16), and α1-antitrypsin (18). It is unclear whether the discrepancies among studies are related to differences with respect to inflammatory markers, study populations, or other factors.

Previous substudies from the Malmö Preventive Study have shown that the risk of developing cardiovascular diseases, hypertension, or a large weight gain increases cumulatively with elevated levels of five inflammation-sensitive plasma proteins (ISPs; fibrinogen, α1-antitrypsin, haptoglobin, ceruloplasmin, and orosomucoid [α1-acid-glucoprotein]) (23–25). The present cohort study explored the relation among C3 and C4, five other markers of inflammation, and the incidence of diabetes.

RESEARCH DESIGN AND METHODS

Between 1974 and 1984, 22,444 men participated in a screening program for the detection of individuals at high risk for cardiovascular diseases (26). The participation rate was 71%. Determination of fibrinogen, α1-antitrypsin, haptoglobin, ceruloplasmin, and orosomucoid levels was part of the program for 6,193 men selected at random from cohorts examined between 1974 and 1982. After men with diabetes or a history of myocardial infarction, stroke, or cancer (determined by questionnaire) were excluded, 5,729 men remained. Information on C3 and C4 was available for 5,530 and 5,528 men, respectively.

A follow-up examination was performed after a mean follow-up of 6.1 ± 0.93 years (range 3.0–9.0 years). Only men born in 1926–1931 or in 1938 (age 38–50 years at baseline) were invited to the follow-up. Of the 3,482 men in these age cohorts who were alive in 1982 when the reexamination started, 2,822 (81%) participated; 7 were subsequently excluded due to missing fasting glucose at the follow-up examination. The sample of the present study thus consisted of 2,815 healthy men, age 38–50 years at baseline, who were reexamined after a mean period of 6.1 years.

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ASP, acylation-stimulating protein; CRP, C-reactive protein; HOMA-IR, homeostasis model assessment for insulin resistance; IL, interleukin; ISP, inflammation-sensitive plasma protein.

The health service authority of Malmö approved the screening program. All participants gave informed consent.

Baseline examinations. Information on fasting glucose was available for all men at both baseline and follow-up. A 2-h oral glucose tolerance test (glucose load: 30 g/m^2 body surface area) was performed in 2,450 men (87%) at the baseline examination and in 2,736 men (97%) at the follow-up examination. Men with fasting whole blood glucose ≥6.1 mmol/l, 2-h post glucose load ≥10.0 mmol/l, or who confirmed their diabetes diagnosis on a self-administered questionnaire were considered to have diabetes.

Information about fasting insulin was available in a subgroup of 1,963 men. Insulin (mIU/l) was measured with a nonspecific radioimmunoassay (27). The homeostasis model formula of Matthews et al. (28), (fasting insulin × fasting glucose) ÷ 22.5, was used to calculate a score for insulin resistance (HOMA-IR).

Subjects were categorized as smokers or nonsmokers. Men with a diabetic father, mother, or sibling were considered to have heredity for diabetes.

Physical inactivity was assessed in a questionnaire at baseline and follow-up. Men who reported that they were mostly sedentary in their spare time were categorized as being physically inactive. Some items of the questionnaire were changed before the end of the follow-up period; for the follow-up examination, physical inactivity was altered to mean those who were mostly sedentary in their spare time or reported that they did not perform physical activity in their spare time (e.g., walking, cycling) regularly every week.

Weight increase (in kilograms) was calculated as weight at baseline subtracted from the follow-up value.

Inflammation-sensitive plasma proteins. An electroimmunoassay was used to assess concentrations of plasma proteins (29). The analysis was performed consecutively at the study entry. The coefficient of variation was <5% (29). Information on C3 and C4 was available for 2,702 men and 2,704 men, respectively. The other proteins were available for all 2,815 men. In accordance with the previous studies from this cohort (23–25), relations with future diabetes are presented for quartiles of the individual proteins as well as in relation to the number of elevated ISPs (fibrinogen, orosomucoid, α1-antitrypsin, haptoglobin, and ceruloplasmin) in the top quartile.

C3 and C4 were originally expressed as the percentage of the mean values from a reference population of blood donors. The reference values for C3 were 70–130% (0.67–1.29 g/l) and for C4 were 65–170% (0.13–0.32 g/l). To facilitate the interpretation of the C3 and C4 values, the percentages have been converted into g/l (C3: 100% = 0.98 g/l; C4: 100% = 0.20 g/l).

The median (25th, 75th percentiles) were 3.46 g/l (3.0, 4.0) for fibrinogen, 0.80 g/l (0.67, 0.93) for orosomucoid, 1.28 g/l (1.09, 1.47) for α1-antitrypsin, 1.30 g/l (0.90, 1.75) for haptoglobin, 0.30 g/l (0.27, 0.35) for ceruloplasmin, 0.98 g/l (0.85, 1.12) for C3, and 0.24 g/l (0.19, 0.29) for C4.

Statistics. The Mann-Whitney U test and Pearson's χ^2 test were used to compare risk factors in men with and without future diabetes. Spearman correlations tested the relation between two continuous variables. Logistic regression was used to study the proportion with incident diabetes in relation to the plasma proteins.

RESULTS

Correlations between C3 and other risk factors. C3 was positively correlated with HOMA-IR (r = 0.20) and BMI (r = 0.32) (Table 1). Other plasma proteins showed smaller correlations with HOMA-IR and BMI.

Diabetes at follow-up. A total of 123 men had developed diabetes at follow-up: 107 had fasting glucose ≥6.1 mmol/l, 45 had 2-h glucose ≥10.0 mmol/l, and 12 had diabetes according to a self-reported questionnaire; 34 met both the fasting glucose and the 2-h glucose criteria. None of them required insulin treatment at the time of examination.

Table 2 presents the baseline characteristics of men who did and did not have diabetes at follow-up. C3, C4, orosomucoid, fibrinogen, and the number of elevated ISPs were significantly higher in men who subsequently developed diabetes.

C3 and incidence of diabetes. The relation between C3 and incidence of diabetes is presented in Table 3, with adjustments in four models. C3 remained significantly associated with diabetes after adjustments for log HOMA-IR or log insulin and for inflammatory markers. When C3 was used as a continuous variable, the adjusted

TABLE 1
Spearman correlations between metabolic parameters and plasma proteins

	Glucose	Insulin	HOMA	BMI	Cholesterol	Triglycerides	Haptoglobin	α1-antitrypsin	Orosomucoid	Ceruloplasmin	C3	C4
Fibrinogen	0.05	0.05	0.06	0.10	0.20	0.15	0.42	0.31	0.37	0.33	0.25	0.19
Haptoglobin	0.14	0.01	0.03	0.01	0.04	0.13	—	0.28	0.42	0.33	0.21	0.23
α1-Antitrypsin	−0.04	0.00	−0.01	−0.11	0.05	−0.01	—	—	0.33	0.31	0.01	0.08
Orosomucoid	0.08	0.02	0.03	0.09	0.13	0.19	—	—	—	0.53	0.29	0.32
Ceruloplasmin	0.01	−0.02	−0.01	0.00	0.14	0.04	—	—	—	—	0.19	0.23
C3	0.19	0.17	0.20	0.32	0.15	0.26	—	—	—	—	—	0.47
C4	0.17	0.00	0.02	0.11	0.10	0.16	—	—	—	—	—	—

r > 0.05 is statistically significant, P < 0.01. Fasting insulin was available in 1,963 men.

TABLE 2
Baseline characteristics of initially nondiabetic men with and without diabetes at follow-up

	No diabetes	Diabetes	*P*
n	2,692	123	—
Age at screening (years)	47.4 ± 2.5	47.9 ± 1.5	0.10
Follow-up time (years)	6.2 ± 0.9	6.2 ± 0.9	0.70
BMI (kg/m^2)	24.6 ± 3.1	27.2 ± 3.9	<0.001
Glucose (mmol/l)	4.7 ± 0.54	5.1 ± 0.58	<0.001
Log HOMA	0.53 ± 0.73	1.01 ± 0.83	<0.001
Cholesterol (mmol/l)	5.7 ± 1.0	5.9 ± 1.0	0.16
Triglycerides (mmol/l)	1.5 ± 0.8	1.9 ± 1.0	<0.001
Smokers (%)	47	49	0.63
Blood pressure medication (%)	4.2	8.8	0.01
Heredity for diabetes (%)	4.1	6.3	0.05
Inflammation-sensitive proteins			
α1-Antitrypsin (g/l)	1.28 ± 0.27	1.30 ± 0.27	0.63
Ceruloplasmin (g/l)	0.312 ± 0.06	0.313 ± 0.06	0.97
Fibrinogen (g/l)	3.47 ± 0.79	3.61 ± 0.76	0.02
Haptoglobin (g/l)	1.29 ± 0.65	1.34 ± 0.56	0.11
Orosomucoid (g/l)	0.82 ± 0.20	0.86 ± 0.20	0.02
Inflammation-sensitive proteins in top quartile (%)			
0	43	28	
1	26	29	
2	14	20	
3 or more	17	22	Trend 0.003
Complement factors			
C3 (g/l)	0.98 ± 0.21	1.12 ± 0.22	<0.0001
C4 (g/l)	0.246 ± 0.08	0.266 ± 0.08	0.008

Data are means ± SE, unless otherwise noted. C3 and C4 were available in 2,702 and 2,704 men, respectively, 117 of whom had incident diabetes.

odds ratio (OR; per 1 SD) was 1.70 (95% CI 1.41–2.04, $P < 0.0001$) after adjustments in model 1 and 1.37 (1.03–1.8, $P = 0.03$) after adjustments in model 4.

The relation between C3 and incidence of diabetes was similar in men with BMI above and below the median (24.4 kg/m^2). The adjusted OR (per 1 SD, model 2) was 1.5 (95% CI 1.03–2.2) for men with BMI below the median (n = 1,350; 32 cases) and 1.5 (1.2–1.9) for men with BMI above the median (n = 1,352; 85 cases).

C3 in relation to BMI and HOMA-IR. C3 was positively associated with HOMA-IR and BMI, two major risk factors for diabetes (Table 1). BMI and HOMA-IR were both strongly associated with incidence of diabetes. The increased risk of diabetes in men with high BMI or HOMA-IR was somewhat weakened after adjustments for C3 but remained significant (Table 4).

C4 and other inflammatory markers. Table 5 presents the ORs of developing diabetes in quartiles of C4 and other inflammatory markers. No significant relation was observed after adjusting for traditional risk factors of diabetes.

Incidence of diabetes was also studied in relation to the number of elevated ISPs (fibrinogen, orosomucoid, α1-antitrypsin, haptoglobin, and ceruloplasmin). After adjusting for age, glucose, and screening year, the adjusted ORs were 1.00, 1.7 (95% CI 1.1–2.8), 2.3 (1.4–4.1), and 1.8 (1.1–3.1), respectively, for men with 0, 1, 2, and 3 or more ISPs in the top quartile (trend: $P = 0.009$). After further adjustment for possible confounders (model 2), this was not significant ($P = 0.07$).

DISCUSSION

Cross-sectional studies have demonstrated strong relations between C3 and various measures of insulin resis-

TABLE 3
Prevalence and odds of diabetes at follow-up in quartiles of C3 at baseline

	Q1	Q2	Q3	Q4	*P* for trend
C3 (g/l)	<0.84	0.85–0.97	0.98–1.12	>1.12	—
n	755	574	686	687	—
Diabetes at follow-up (%)	1.5	3.3	4.2	8.4	<0.0001
Model 1	1.00	2.4 (1.1–5.3)	2.9 (1.4–6.0)	5.6 (2.8–10.9)	<0.0001
Model 2	1.00	2.2 (1.0–4.7)	2.3 (1.1–4.8)	3.8 (1.9–7.7)	<0.001
Model 3	1.00	2.1 (0.83–5.4)	2.6 (1.1–6.1)	3.3 (1.4–7.8)	0.006
Model 4	1.00	2.1 (0.80–5.3)	2.5 (1.1–6.2)	3.2 (1.3–8.0)	0.017

Data are OR (95% CI), unless otherwise noted. In analysis for models 3 and 4, 1,907 men, 87 with future diabetes, were available. Model 1: Odds ratios adjusted for age, screening year, and glucose at baseline. Model 2: Adjusted as in model 1 plus heredity for diabetes, smoking, triglycerides, blood pressure−lowering medication, BMI, weight increase during follow-up, and physical activity at baseline and follow-up. Model 3: Adjusted as in model 2 plus log HOMA. Model 4: Adjusted as in model 3 plus fibrinogen, haptoglobin, orosomucoid, α1-antitrypsin, ceruloplasmin, and C4.

TABLE 4
Prevalence and odds of diabetes at follow-up in relation to quartiles of BMI and HOMA-IR at baseline

	Diabetes at follow-up (%)	Adjusted for age and screening year	Adjusted for age, screening year, and C3
BMI			
Q1 (<22.6)	1.2	1.00	1.00
Q2 (22.6–24.4)	3.6	3.0 (1.3–6.7)	2.5 (1.1–5.7)
Q3 (24.4–25.8)	3.4	2.8 (1.2–6.3)	2.3 (1.0–5.1)
Q4 (>25.8)	9.2	8.2 (3.9–17)	5.5 (2.6–12)
HOMA			
Q1 (<0.91)	2.9	1.00	1.00
Q2 (0.91–1.8)	2.3	0.8 (0.4–1.8)	0.8 (0.4–1.7)
Q3 (1.8–3.0)	4.6	1.7 (0.9–3.5)	1.5 (0.7–2.9)
Q4 (>3.0)	8.3	3.5 (1.8–6.6)	2.5 (1.3–4.9)

Data are OR (95% CI), unless otherwise noted. For HOMA, 1,907 men, 87 with future diabetes, were available for analysis.

tance. This prospective study showed that C3 was associated with a markedly increased risk of developing diabetes, which remained after adjustments for HOMA-IR, BMI, and several markers of inflammation. To the best of our knowledge, this is the first study of the incidence of diabetes in relation to C3.

Previous studies about inflammation and incidence of diabetes have shown quite different relations for different inflammatory markers. In the present study, the relation between C3 and diabetes was substantially stronger than for other inflammatory markers and diabetes, and only C3 was significantly associated with diabetes after adjusting for potential confounders. In agreement with Schmidt et al. (18), no significant relation was found for α1-antitrypsin. The relations with haptoglobin and fibrinogen were nonsignificant after adjustments, which concurs with the studies of Festa et al. (22) and Schmidt et al. (18).

C3 showed moderate correlations with HOMA-IR and BMI, whereas the other plasma proteins were essentially unrelated to HOMA-IR. However, the relation between C3 and incidence of diabetes persisted after adjustments for HOMA-IR and BMI. This suggests that C3 may be linked to diabetes through other pathways as well. C3 is mainly produced in the liver in response to proinflammatory cytokines (e.g., IL-6 and IL-1) (1,2). High gene expression of C3 is also observed in omental adipose tissue (3). Adipose tissue is an organ with endocrine functions, and proinflammatory cytokines formed in adipose tissue have been associated with impaired glucose uptake and insulin resistance (21,30–33). The relation between C3 and incidence of diabetes could reflect a systemic low-grade inflammation and the actions of these cytokines.

ASP, the proteolytic fragment of C3, is another possible link between C3 and diabetes. ASP stimulates glucose uptake and lipid storage in adipose tissue (10–12). ASP deficiency in mice has been associated with reduced weight (13), and high ASP levels have been reported in nondiabetic subjects with insulin resistance (9,34). The reduced uptake of glucose and fatty acids in the adipose tissue of diabetic subjects could be related to a blunted response to activation of the C3-ASP system. However, this hypothesis is challenged by the fact that C3-knockout mice have shown only modest alterations in insulin function and glucose metabolism (35).

The relation between diabetes and inflammation could also be associated with hepatic production of glucose. Type 2 diabetes is associated with abnormalities in hepatic glucose production (36). Most blood glucose and plasma proteins originate from the liver, and the synthesis is regulated by, for example, IL-6 and other cytokines (2,37,38). Dysregulation of hepatic glucose production in combination with insulin resistance could contribute to the relation between inflammatory proteins and diabetes.

In this study, blood glucose was measured only once at both baseline and follow-up. As men with whole blood glucose ≥6.1 mmol/l were considered diabetic, it is possible that some were false-positive cases. However, C3 was associated with diabetes even when a higher cutoff was used (≥6.7 mmol/l; data not shown).

The relation between C3 and diabetes was observed in men with BMI above and below the median (24.4 kg/m^2). Diabetes onset in men with normal weight is more often related to the presence of autoantibodies and less often with insulin resistance (39). Analysis of autoantibodies was not performed in clinical practice when the men were examined. However, all men were nondiabetic at the first examination, and new cases of type 1 diabetes are unusual in this age-group. A retrospective study of the hospital records indicated that only two subjects developed type 1 diabetes, based on a clinical definition of type 1 diabetes (insulin dependency within 3 years after diabetes diagnosis and no discontinuation of insulin after that). The study results were unchanged after these subjects were excluded. We can conclude that C3 is associated with development of type 2 diabetes in middle-aged men; we cannot make any conclusions about other subtypes of diabetes.

The strengths of this study include the large population-

TABLE 5
Odds ratios of diabetes at follow-up in relation to quartiles of inflammatory proteins at baseline

	Q2	Q3	Q4	*P* for trend (model 1)	*P* for trend (model 2)
Fibrinogen	0.97 (0.5–1.7)	1.1 (0.6–2.0)	1.6 (0.97–2.8)	0.04	0.26
Orosomucoid	2.1 (1.2–3.8)	1.5 (0.8–3.1)	2.1 (1.2–3.8)	0.07	0.50
Haptoglobin	1.4 (0.8–2.5)	1.7 (0.96–2.9)	1.7 (0.96–3.1)	0.05	0.15
Ceruloplasmin	1.2 (0.7–2.0)	1.0 (0.6–1.7)	1.2 (0.6–2.1)	0.86	0.84
α1-Antitrypsin	1.6 (0.92–2.7)	1.1 (0.6–2.1)	1.3 (0.8–2.2)	0.66	0.70
C4	1.7 (0.8–3.4)	2.1 (1.1–4.0)	2.6 (1.4–5.2)	0.003	0.08

Data are OR (95% CI). Model 1: OR and *P* for trend adjusted for age, screening year, and glucose at baseline. Model 2: As in model 1 plus heredity for diabetes, smoking, triglycerides, blood pressure−lowering medication, BMI, weight increase during follow-up, and physical activity at baseline and follow-up. Q1 was used as reference category.

based cohort and the presence of information on fasting glucose, 2-h glucose, insulin, and several markers of inflammation. The participation rates were high and the same procedures were used at both baseline and follow-up examinations. The analyses were, however, limited to those measurements that were available in clinical practice when the study began. For example, it was not possible to analyze high-sensitivity CRP, tumor necrosis factor-α, or IL-6 at the time of the baseline examination, which is a limitation of the study.

No information about the waist-to-hip ratio was available either. However, the results were adjusted for BMI and triglyceride levels at baseline and weight increase during follow-up. The correlations between C3 and BMI are similar and sometimes stronger than correlations between C3 and waist-to-hip ratio (6,9,34). Thus, it can be assumed that the adjustments for body fat were fully adequate in this study.

Apart from cytokine stimulation of C3 synthesis, C3 concentrations are determined by immune-complex deposition, genetic factors, and female sex hormones (1). Whether the relation between C3 and incidence of diabetes is similar in women remains to be explored.

In conclusion, in this study, elevated levels of C3 were associated with an increased incidence of diabetes in middle-aged men. This association remained significant after adjustments for BMI, HOMA, and other markers of inflammation.

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