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Engström, Gunnar; Hedblad, Bo; Janzon, Lars; Lindgärde, Folke

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**Weight gain in relation to plasma levels of complement C3. Results from a
population-based cohort study.**

(short title: G Engström et al: C3 and weight gain)

Authors: G Engström, B Hedblad, L Janzon, F Lindgärde

**From the Department of Clinical Sciences, Lund University, Malmö
University Hospital, Malmö, Sweden.**

Correspondence: Gunnar Engström, PhD, Department of Clinical Sciences, MFC
Ing 59, Malmö University Hospital, S-20502 Malmö, Sweden.
Phone: +46-40332670, Fax: +46-40336215, Email:
Gunnar.Engstrom@smi.mas.lu.se

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Abstract

Aims/Hypothesis Mice with deficiency of complement C3 have shown resistance to weight gain, despite an increased food intake. Cross-sectional studies of humans have reported correlations between C3 and obesity. This longitudinal study explored whether C3 predicts a large weight gain in middle-aged men.

Methods Plasma concentrations of C3 and C4 was measured in 2706 non-diabetic healthy men, 38-50 years, who were re-examined after a mean period of 6.1 years.

Results After adjustments for initial weight, age, height and follow-up time, the odds of developing a large weight gain (75th percentile, ≥ 3.8 kg) was 1.00 (reference), 0.96 (95% CI:0.7-1.2), 1.1 (CI:0.9-1.5) and 1.4 (CI:1.1-1.8), respectively, among men with C3 in the 1st, 2nd, 3rd and 4th quartile (p for trend=0.010). This relationship remained significant after further adjustments for life style factors (physical inactivity, alcohol, smoking), metabolic factors (glucose or HOMA-values, cholesterol, triglycerides), inflammatory markers (fibrinogen, haptoglobin, ceruloplasmin, orosomucoid, $\alpha 1$ -antitrypsin) and C4.

C4 was associated with weight gain after adjustments for initial weight, height, follow-up time and life style factors, but not after adjustments for C3.

Conclusions/Interpretation C3 is a risk factor for developing a large weight gain in middle-aged men.

Key words: Complement, obesity, epidemiology

Abbreviations

HOMA; homeostasis model assessment

C3 and C4; complement factors 3 and 4

BMI; body mass index

ISPs; inflammation sensitive plasma proteins

OR; odds ratio

Q; quartile

SD; standard deviation

SE; standard error

Introduction

C3 and C4 are the major plasma proteins of the complement pathway of the immune system. The synthesis of these proteins is increased in response to inflammation and infection, with a slower increase than the traditional acute phase proteins (1-3). Although the hepatic production is the main source, C3 and C4 are also expressed and secreted by other cells, for example, in the adipose tissue (4,5).

Individuals with overweight or obesity generally have raised concentrations of C3 and C4 (4-10). It has been reported that the C3 levels decrease after weight loss (6,7). The proteolytic fragment of C3, acylation-stimulating protein (ASP), is a paracrine metabolic factor that stimulates the uptake of glucose and the fat storage in human adipose tissue (11-14). Knock out mice with deficiency of C3 and ASP show resistance to weight gain on a high-fat diet, despite an increased food intake (11,14). However, no prospective study has investigated the relationship between C3 and weight gain in humans.

Previous studies from the present cohort have shown that raised concentrations of fibrinogen, haptoglobin, ceruloplasmin, orosomucoid and α 1-antitrypsin are associated with weight gain (15). The correlations between C3 and these inflammatory markers are small, however, and we recently reported that C3 is associated with incidence of diabetes independently of these proteins (16). Based on the findings from animal models, we explored the hypothesis that C3 could be associated with future weight increase independently of other inflammatory markers.

Subjects and Methods

Between 1974 and 1984, 22444 men participated in a screening program for detection of high risk for cardiovascular diseases. Complete birth cohorts from the city of Malmö were invited (17). Participation rate was 71%. Determination of plasma proteins was part of the program for 6193 men, selected at random from cohorts examined between 1974 and 1982. After the exclusion of men with diabetes or a history of myocardial infarction, stroke or cancer (according to questionnaire), 5729 men remained.

A follow-up examination was performed after a mean follow-up of 6.1 ± 0.93 years (Range: 3.0 to 9.0). Of the screened birth cohorts, men born in 1926-1931 and 1938 (38-50 years old at baseline) were invited to the follow-up. A total of 2822 participated, which was 81% of those who were alive in 1982 when the re-examination started. Of them, C3 was available for 2706 and C4 for 2704 men. The sample of the present study thus consists of 2706 healthy men, 38-50 years old at baseline, who were re-examined after a mean period of 6.1 years.

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

Baseline examinations

Diabetes was defined as fasting whole blood glucose ≥ 6.1 mmol/l, 2h glucose ≥ 10.0 mmol/l (glucose load: 30 g/m² body surface area), or self-reported diabetes.

Information about fasting insulin was available in a subgroup of 1907 men. Insulin (mIU/l) was measured with a non-specific radioimmunoassay (18). The homeostasis model formula according to Matthews et al (19), fasting insulin x

fasting glucose/22.5, was used to calculate a score for insulin resistance (HOMA).

Subjects were categorised into smokers and non-smokers. Physical inactivity was assessed in a questionnaire at baseline and at follow-up. Men who reported that they mostly were sedentary in spare time were categorised as physically inactive. Some items of the questionnaire were changed before the end of the follow-up period. At the follow-up examination, physical inactivity was therefore defined either as those who mostly were sedentary in spare time or those who reported that they did not perform physical activity in spare time (e.g. walking, cycling) regularly every week. Alcohol consumption was assessed by means of the modified shortened version of the Michigan Alcoholism Screening Test (20). Men with more than two (of nine) affirmative answers were considered to be high-consumers of alcohol.

Weight was measured in the morning with the subject wearing light indoor clothing and no shoes. Weight increase (in kg) was calculated as weight at baseline subtracted from the follow-up value. A large weight gain was defined as the 75th percentile (>3.8 kg) (15).

Plasma proteins

Electroimmuno assay was used to assess the concentrations of plasma proteins (21). The coefficient of variation was <5% (21). The analysis was performed consecutively at the study entry. 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%, which corresponds to 0.67-1.29 g/l, and the reference values for C4 were 65-170% (0.13-0.32 g/l). In order 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 distributions of the other plasma proteins (fibrinogen, haptoglobin, ceruloplasmin, orosomucoid, α 1-antitrypsin) have been presented elsewhere (22). The C3-C4 correlation was $r=0.47$ and the correlations between C3 and the other proteins were below 0.3 in this cohort (16).

Statistics

One-way analysis of variance and the Mantel-Haenzel chi-square test were used to compare the risk factors by quartiles of C3. Pearson's correlation was used for two continuous variables. A general linear model was used to compare weight at follow-up in quartiles of C3, with adjustments for confounding factors. Logistic regression was used to study the proportion with large weight gain in relation to plasma proteins. The relationships between C3 and weight gain were adjusted for initial weight in all calculations. Two-tailed p-values <0.05 were considered statistically significant.

Results

The baseline and follow-up characteristics by quartiles of C3 are presented in Table 1. BMI and weight were substantially higher in men with high C3. The proportion of smokers was somewhat lower in men with high C3 (Table 1). However, C3 showed no association with smoking after adjustments for BMI (data not shown).

Weight at baseline was inversely correlated with weight increase during the follow-up ($r=-0.17$, $p<0.0001$). The proportion with a large weight gain (>3.8 kg) was 24.1% in men with low C3 (Q1), and 25.9% in men with high C3 (Q4). However, when weight at baseline, age, height and follow-up time were taken

into account, a large weight gain was significantly associated with C3 at baseline (Table 2). This relationship remained significant after further adjustments for life style factors (Model 2: +smoking, physical activity, alcohol consumption), metabolic factors (Model 3: +glucose, cholesterol, triglycerides), and inflammation-sensitive proteins (ISPs) (Model 4: +fibrinogen, ceruloplasmin, α 1-antitrypsin, orosomucoid, haptoglobin). The results also remained significant after adjustments for C4. The relationships were similar when C3 was used as a continuous variable (per SD) (Table 2).

Weight at follow-up showed similar associations with C3 after adjustments for initial weight (Table 2). C3 was significantly associated with weight at follow-up after adjustments for inflammatory markers (Model 4). After further adjustments for C4, this association was borderline significant ($p=0.06$).

Weight loss >1.2 kg (lowest quartile of weight gain) was not associated with C3 after adjustment for initial weight (Table 2).

The results were identical if weight increase (initial weight subtracted from weight at follow-up) was used instead of weight at follow-up. The results were also identical if BMI was used to assess weight and weight increase (not shown). The results were essentially unchanged after exclusion of 117 subjects who developed diabetes during the follow-up period (not shown).

The relationships between C3 and weight gain were largely consistent over the quartiles of BMI. The relationships were somewhat weaker in smokers than in non-smokers or smokers who quit during the follow-up period (Table 3).

Subgroup with HOMA-values

Information about HOMA was available for a subgroup of 1907 men. The relationship with large weight gain was studied in this subgroup, with and without adjustments for HOMA (log transformed). After adjustments for initial weight, age, height, follow-up time, life style, and metabolic factors (Model 3), the odds ratios for a large weight gain were 1.00, 1.1 (0.8-1.6), 1.2 (0.9-1.7) and 1.56 (1.1-2.2), respectively, for C3 in 1st, 2nd, 3rd, and 4th quartile (p for trend=0.008). OR per 1 SD increase in C3 was 1.17 (1.04-1.32, p=0.007). When HOMA was added to the model, the ORs were 1.00, 1.1 (0.8-1.6), 1.2 (0.9-1.7), and 1.54 (1.1-2.1), respectively (p for trend: 0.009). OR per 1 SD was 1.17 (1.04-1.31, p=0.01). These relationships remained significant after further adjustments for ISPs (Model 4).

The relationship between C3 and weight at follow-up was significant after adjustments for life style, metabolic factors (Model 3) and HOMA (adjusted mean + standard error, 78.1+0.20, 78.3+0.22, 78.3+0.20 and 78.8+0.21 kg, respectively, for the quartiles of C3, p for trend<0.05). After further adjustments for inflammatory proteins, this association did not reach significance in this subgroup (p for trend=0.11).

Inflammation-sensitive plasma proteins (ISPs) and C4

Table 4 presents the relationships between a large weight gain and the number of ISPs in the top quartile. The risk of a large weight gain was significantly associated with the number of elevated ISPs, and this relationship remained significant after adjustments for C3 and C4.

Table 5 presents the relationship between C4 and a large weight gain. C4 was associated with weight gain after adjustments for initial weight, follow-up time and life style factors. However, there was no association between C4 and weight gain after adjustments for C3. This relationship was similar when C4 was used as a continuous variable (not shown).

Discussion

Cross-sectional studies have shown that obesity is associated with elevated plasma levels of C3. The causal and temporal relations of this association have been unclear. The present study showed that C3 was strongly associated both with weight at baseline and weight at follow-up. When adjusting for initial weight, C3 was significantly associated with an increased risk of developing a large weight gain. To our knowledge, this is the first study linking C3 to future weight gain in humans.

Although the causes of obesity are multifactorial, increasing evidence links inflammation and infections to obesity or weight gain (23-28). Seropositivity for *Chlamydia pneumoniae* has been associated with high BMI in humans (28) and virus infections in mice have been associated with weight gain (27). Elevated levels of fibrinogen, white blood cells, von Willebrand factor or factor VIII, i.e. four putative markers of inflammation, have been associated with large weight gain in a large cohort study (26). A study from this cohort showed that high levels of fibrinogen, ceruloplasmin, α 1-antitrypsin, orosomucoid and haptoglobin were associated with weight gain (15). However, C3 shows only modest correlations with these inflammatory markers (all $r < 0.3$) (16). The relationship between C3

and weight gain was essentially unchanged after adjustment for inflammatory proteins and, conversely, the number of elevated ISPs was associated with weight gain even after adjustments for C3. Hence, the relationship between C3 and weight gain cannot be explained by inflammation as measured by these acute phase proteins. C3 and the ISPs could represent different pathways of weight gain.

Another question is whether the relationships are specific for C3 or whether other complement components are associated with weight gain as well. Data on C4 was available in this study and the correlation between C3 and C4 was approximately $r=0.5$. The relationship between C3 and weight gain was slightly reduced after adjustments for C4. In contrast, the relationship between C4 and weight gain became clearly non-significant after adjustment for C3. Hence, the association between C4 and weight gain seems to be explained by its relationship with C3.

ASP is a possible link between C3 and weight gain. This hormone has autocrine or paracrine effects and is produced in the adipose tissue by conversion from C3 (11). It has been shown that ASP stimulates the fat storage in human adipocytes, inhibits the intracellular lipolysis and increases plasma clearance of triglycerides (11-13). ASP also stimulates the glucose disposal by increasing the release of insulin (29). Mice with deficiency of C3 and ASP show resistance to weight gain despite an increase food intake (14). These animals also have increased energy expenditure (30). However, it is unclear to what extent circulating C3 reflects the concentrations in the adipose tissue. As local effects in the adipose tissue

probably are most important, circulating levels of C3 could be a crude proxy for the local effects.

The hepatic synthesis of C3 is stimulated by various cytokines, e.g., IL-1 and TNF- α (31). These cytokines are also involved in the regulation of metabolism and food intake. For example, TNF- α and IL-1 β decrease the production and expression of leptin, an anorexogenic hormone formed in the adipose tissue (32). Genetic polymorphisms of the TNF- α receptor 2 gene and the -308A variant of the TNF- α gene have been associated with leptin-resistance and obesity (33,34). In studies of rodents, injection of TNF- α and IL-1 initially reduce the food intake. However, tolerance soon develops after repetitive administration of TNF- α (35). The relations between plasma C3 and weight gain could hypothetically reflect the effects of these cytokines.

We had no information on diet in this cohort. It is unclear whether the relations between weight and C3 could reflect dietary factors that increase C3. It has been reported that intake of lipids and proteins could induce oxidative stress (36), which hypothetically could increase inflammation. On the other hand, studies of C3 before and after food intake have not shown any consistent differences. Two studies reported no post-prandial changes (37,38), while another study showed a small increase (39). Since the plasma samples were drawn after an overnight fast, this probably reduced the effects of food intake.

The participant rates were high and the procedures were identical at both examinations. As smoking cessation is associated with weight gain, smoking is a

potential confounder. However, smoking was not associated with C3 after body weight had been taken into account and the relation between C3 and weight gain persisted after adjustments for current and former smoking and smoking cessation during follow-up. Physical activity is another factor that could affect weight gain. The results were adjusted for physical inactivity both at baseline and at follow-up. However, only two categories of physical activity were used and it is possible that the variables did not pick-up all effects of physical activity. Another limitation is that only men were included in the study. Apart from the cytokine stimulation of the C3 synthesis, the C3 concentrations are determined by immune-complex deposition, genetic factors and female sex hormones (40). Whether the relation between C3 and weight gain is similar in women is unknown.

It is concluded that high levels of complement C3 is a risk factor for developing a large weight gain. The reasons for this association remain to be explored.

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Table 1. Description of the study cohort at baseline and at follow-up in relation to quartile of C3 at baseline.

	C3 quartile				P for trend
	Q1	Q2	Q3	Q4	
C3 (g/l)	≤0.84	0.85-0.97	0.98-1.12	>1.12	
N	756	574	690	686	
Baseline characteristics					
Age at baseline (years)	47.3±2.5	47.4±2.6	47.4±2.4	47.3±2.5	0.79
Smokers (%)	48	51	45	44	0.04
High alcohol consumption (%)	9.4	8.7	7.2	10.1	0.94
Physical inactivity (%)	56	50	55	61	0.03
BMI (kg/m ²)	23.5±2.7	24.3±3.0	25.1±3.0	26.2±3.5	<0.001
Height (cm)	176.8±6.7	176.7±6.7	176.2±6.5	175.8±6.2	0.003
Weight (kg)	73.5±10.1	75.8±10.5	77.9±10.4	81.0±11.9	<0.001
Cholesterol (mmol/l)	5.5±1.0	5.7±1.0	5.8±1.0	5.9±1.1	<0.001
Triglycerides (mmol/l)*	1.19	1.32	1.45	1.62	<0.001
Glucose (mmol/l)	4.6±0.5	4.7±0.5	4.7±0.5	4.9±0.6	<0.001
HOMA*	1.44	1.69	1.78	2.10	<0.001
α1-antitrypsin (g/l)	1.27±0.26	1.30±0.27	1.29±0.27	1.29±0.28	0.35
Fibrinogen (g/l)	3.23±0.70	3.46±0.78	3.54±0.77	3.72±0.82	<0.001
Ceruloplasmin (g/l)	0.30±0.06	0.31±0.06	0.32±0.06	0.33±0.06	<0.001
Haptoglobin (g/l)	1.12±0.57	1.27±0.60	1.33±0.63	1.46±0.67	<0.001
Orosomucoid (g/l)	0.75±0.18	0.81±0.19	0.84±0.18	0.89±0.21	<0.001
C4 (g/l)	0.20±0.06	0.23±0.06	0.26±0.07	0.30±0.09	<0.001
Follow-up characteristics					
Follow-up (years)	6.2±0.9	6.3±0.9	6.2±0.9	6.0±0.9	0.004
Smokers (%)	39	40	37	34	0.04
Physical inactivity (%)	51	50	48	53	0.71
BMI (kg/m ²)	24.1±2.8	24.7±3.0	25.5±3.1	26.6±3.6	<0.001

* geometric means.

HOMA values were available for 1907 men.

Table 2. Weight gain in relation to quartiles of C3.

	C3				P for trend	per 1 SD*
	Q1	Q2	Q3	Q4		
g/l	≤0.84	0.85-0.97	0.98-1.12	>1.12		
N	756	574	690	686		
Large weight gain (≥3.8 kg) (%)	24.1	22.8	24.9	25.9		
Adjusted OR, Model 1 ^a	1.00	0.96 (0.7-1.2)	1.1 (0.90-1.5)	1.4 (1.06-1.8)	0.010	1.14 (1.04-1.26)
Model 2 ^b	1.00	0.92 (0.7-1.2)	1.2 (0.90-1.5)	1.4 (1.05-1.8)	0.009	1.15 (1.04-1.26)
Model 3 ^c	1.00	0.97 (0.7-1.3)	1.2 (0.97-1.6)	1.5 (1.2-2.0)	0.001	1.20 (1.09-1.32)
Model 4 ^d	1.00	0.94 (0.7-1.2)	1.2 (0.92-1.5)	1.4 (1.07-1.8)	0.006	1.17 (1.05-1.30)
+C4 ^e	1.00	0.92 (0.7-1.2)	1.1 (0.88-1.5)	1.3 (0.98-1.8)	0.034	1.14 (1.02-1.28)
Weight at follow-up (mean±SD)	75.1±10.1	77.1±10.5	79.2±10.7	82.2±12.2	<0.0001	
Adjusted mean±SE (kg)						
Model 1 ^a	78.3±0.17	78.2±0.19	78.4±0.17	78.7±0.17	0.07	
Model 2 ^b	78.3±0.16	78.1±0.18	78.4±0.16	78.7±0.17	0.06	
Model 3 ^c	78.1±0.16	78.1±0.18	78.5±0.17	78.8±0.17	0.003	
Model 4 ^d	78.2±0.17	78.1±0.18	78.4±0.17	78.7±0.19	0.02	
+C4 ^e	78.2±0.17	78.1±0.18	78.4±0.17	78.7±0.19	0.06	
Weight loss >1.2 kg (%)	22	25	28	27		
Adjusted OR, model 1 ^a	1.00	0.98 (0.7-1.3)	0.96 (0.7-1.2)	0.90 (0.7-1.2)	0.43	

^a adjusted for age, follow-up time, height, initial weight.

^b + physical inactivity at baseline and follow-up, high alcohol consumption, current and former smoking at baseline, smoking at follow-up and smoking cessation during follow-up.

^c +glucose, triglycerides, cholesterol

^d + fibrinogen, ceruloplasmin, α1-antitrypsin, orosomucoid, haptoglobin

^e + Model 4 +C4

* OR per 1 standard deviation increase in C3 (0.22 g/l)

Table 3. The odds of a large weight gain (>3.8 kg) in relation to C3, by smoking and quartiles of BMI.

BMI	
Q1 (<22.6)	
Large weight gain, n (%)	212 (31)
OR* per 1 SD	1.25 (1.03-1.5)
Q2 (22.6-24.4)	
Large weight gain, n (%)	158 (23)
OR* per 1 SD	1.13 (0.91-1.4)
Q3 (24.4-25.8)	
Large weight gain, n (%)	154 (23)
OR* per 1 SD	1.26 (1.04-1.5)
Q4 (>25.8)	
Large weight gain, n (%)	139 (20)
OR* per 1 SD	1.24 (1.02-1.5)
Smoking status	
Non-smokers	
Large weight gain, n (%)	301 (21)
OR* per 1 SD	1.22 (1.07-1.4)
Continuing smokers	
Large weight gain, n (%)	209 (22)
OR* per 1 SD	1.10 (0.92-1.3)
Smokers who quit during follow-up	
Large weight gain, n (%)	151 (47)
OR* per 1 SD	1.28 (0.98-1.7)

*Odds ratio per 1 standard deviation of C3 (0.22 g/l), adjusted for age, follow-up time, initial weight, height, physical inactivity at baseline and follow-up, high alcohol consumption, cholesterol, glucose, log triglycerides, current and former smoking at baseline, smoking at follow-up and smoking cessation during follow-up (smoking only used in quartile of BMI).

Table 4. Weight gain in relation to inflammation-sensitive plasma proteins (ISPs).

	Number of elevated ISPs†				P for trend
	None	1	2	3 or more	
N	1139	712	385	470	
Initial weight (kg)	76.9+10.9	77.4+11.0	76.9+11.5	76.5+11.4	0.53
Large weight gain (≥ 3.8 kg) (%)	20.9	26.5	26.2	28.7	
Adjusted OR* ^a	1.00	1.39 (1.1-1.7)	1.41 (1.06-1.9)	1.59 (1.2-2.1)	0.001
+ C3 and C4	1.00	1.34 (1.1-1.7)	1.31 (0.98-1.8)	1.41 (1.1-1.9)	0.013

† fibrinogen, ceruloplasmin, α 1-antitrypsin, orosomucoid or haptoglobin in the 4th quartile

*adjusted for age, follow-up time, initial weight, height, physical inactivity at baseline and follow-up, high alcohol consumption, current and former smoking at baseline, smoking at follow-up and smoking cessation during follow-up, glucose, triglycerides, cholesterol

Table 5. Weight gain in relation to quartiles of C4.

	C4				P for trend
	Q1	Q2	Q3	Q4	
g/l	<0.19	0.19-0.24	0.24-0.29	>0.29	
N	680	778	579	667	
Initial weight (kg)	75.6+10.6	76.9+10.4	78.1+11.8	77.6+11.5	<0.001
Large weight gain (≥ 3.8 kg) (%)	22.4	26.2	23.5	25.6	
Risk factor adjusted OR ^{*a}	1.00	1.36 (1.06-1.8)	1.20 (0.9-1.8)	1.50 (1.1-2.0)	0.01
Risk factors + C3	1.00	1.29 (1.0-1.7)	1.10 (0.8-1.5)	1.27 (0.9-1.7)	0.26
Risk factors + C3 and ISPs ^{**}	1.00	1.28 (1.0-1.7)	1.08 (0.8-1.4)	1.24 (0.9-1.7)	0.37

*adjusted for age, follow-up time, weight at baseline, height, physical inactivity at baseline and follow-up, high alcohol consumption, current and former smoking at baseline, smoking at follow-up and smoking cessation during follow-up, glucose, triglycerides, cholesterol

** fibrinogen, ceruloplasmin, α 1-antitrypsin, orosomucoid, haptoglobin

