Relationship between C3 Levels and Common Carotid Intima-Media Thickness in Overweight and Obese Patients

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Keywords

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Summary

Objective: The study aim was to compare C3 levels with the common carotid artery intima-media thickness (CCA-IMT) in subjects of both genders, with a wide range of BMI, independently of age, gender, and abdominal obesity. **Method:** 140 euthyroid, mainly overweight/obese subjects (age 18–30 years) were examined. BMI, waist circumference, blood pressure, fasting insulin, glucose, lipids, C3 and C-reactive protein serum concentrations, and insulin resistance degree (estimated by homeostasis model assessment for insulin resistance (HOMA_{IR})) were measured. **Results:** CCA-IMT was positively ($p < 0.001$) correlated with BMI, waist circumference, systolic and diastolic blood pressures, HOMA_{IR}, and insulin, CRP, and C3 serum levels. The multiple linear regression analysis showed that only male gender and waist circumference maintained an independent relation with the CCA-IMT. **Conclusion:** This study suggests that central fat accumulation and male gender independently increase the thickness of the arterial wall, whereas inflammation and inflammatory markers do not have an independent effect on this parameter.

Introduction

The atherosclerotic process is now considered a chronic inflammatory process in which immune mechanisms with metabolic risk factors interact to initiate and develop vascular lesions [1]. The complement is a system of proteins functionally interacting with each other in order to provide many of the effector functions of humoral immunity and inflammation. The central component of the complement system is the complement 3 (C3) fraction, as all the pathways for the activation of the system converge there. It is noteworthy that the presence of C3 was verified in the atherosclerotic plaque [2] and that C3 has been shown to be a strong predictor of myocardial infarction [3].

Adipocytes are an important source of C3 production in addition to that produced in the liver, in response to interleukin(IL)-1, and in activated macrophages. It has been shown that adipose tissue produces all the factors of the alternative pathway for activation of the complement [4]; recently, the gene expressions that code the proteins activating the complement system have been verified in human adipocytes [5].

There is a correlation between C3 levels and the majority of conventional cardiovascular risk factors in both the general population [3] and patients with cardiovascular disease [6], and also a positive correlation was found between baseline insulin levels and C3 levels [6]. Serum C3 could even be considered as a stronger inflammatory marker of insulin resistance than C-reactive protein (CRP), leukocyte count, and erythrocyte sedimentation rate [7]. On the other hand, the concentrations of C3 positively correlate with visceral and

Fig. 1. Flow chart of the study.

subcutaneous fat [4] and BMI [4, 5] in the general population of both genders. A recent study has also shown a relationship between C3 and the progressive increase of BMI in subjects with severe, morbid, or extreme obesity, and furthermore that the increase in C3 was closely related to insulin levels and insulin resistance (measured by homeostasis model assessment for insulin resistance, HOMA_{IR}) [8].

It has been shown that the adipocytes express tumor necrosis factor α (TNF- α) and may produce 30–40% of the circulating levels of IL-6 [9], the main regulator of CRP production in the liver. Therefore, the hyperproduction of cytokines is associated with a condition of low-grade chronic inflammation, which is responsible for the activation of the complement system; a situation that could contribute to the metabolic complications observed in obesity [10].

It has been shown that atherosclerosis is accelerated in obese subjects, and in those with central obesity in particular, as shown by higher common carotid artery intima-media thickness (CCA-IMT) [11–17]. In fact, the CCA-IMT is considered as a marker of initial asymptomatic atherosclerosis and could precede the development of plaque and stenosis in the arterial wall [18, 19]. It is noteworthy that the association of central obesity with early carotid intima-media thickening is independent of that from other risk factors [12, 15, 17], and that leptin, a protein quite exclusively produced by adipose tissue, is independently associated with the CCA-IMT [11].

To the best of our knowledge, no study has simultaneously evaluated C3, insulin, glucose and lipid concentrations, blood pressure levels, insulin resistance, and CCA-IMT in a population of overweight and/or obese subjects.

This study considered an evaluation of the C3 levels as a function of the CCA-IMT in subjects of both genders with a range of BMI, independently of obesity and other cardiovascular risk factors. To this aim, a cohort of 140 euthyroid, apparently healthy overweight and obese subjects was investigated.

Material and Methods

Subjects

All subjects (fig. 1) were recruited consecutively at the Outpatient Clinic for the Study of Obesity and Nutrition Diseases, Section of Internal Medicine, Endocrinology, Andrology and Metabolic Diseases, Department of Emergency and Organ Transplantation, University of Bari, School of Medicine, Bari, Italy. The majority of individuals had a BMI > 25.0; however, 13 of them had a BMI < 25.0, and they were referred to the Outpatient Clinic because their weight had recently increased in order to be educated regarding correct food and lifestyle habits.

In our experience, age has a strong influence on the thickening of the intima-media complex, thus limiting the possibility to identify cardiovascular risk factors having a direct effect on the thickening of the arterial wall. Therefore, only young subjects, aged 18–30 years, were enrolled.

To avoid the influence of confounding factors, the following patients were excluded from the study: smokers, subjects taking any kind of drugs, and patients known to be affected by thyroid dysfunction, endocrinological diseases, diabetes mellitus, stable and drug-treated hypertension, stroke, transient ischemic attack, angina pectoris, heart infarction, claudicatio intermittens, congenital heart disease, or ECG abnormalities.

Paying heed to the above exclusion criteria, 140 subjects were finally enrolled, 75% women (n = 105) and 25% (n = 35) men. All subjects gave their informed consent to be included into the study, which was performed in accordance with the guidelines proposed in the Declaration of Helsinki.

Table 1. General, anthropometric, hormonal, and metabolic parameters in subjects under study (n = 140)

Age, years	23 ± 4
Body mass index, kg/m ²	33.7 ± 7.1
Waist circumference, cm	107 ± 15
Systolic blood pressure, mm Hg	118 ± 12
Diastolic blood pressure, mm Hg	75 ± 7
Fasting insulin, μUI/ml	27 ± 16
Fasting blood glucose, mg/dl	86 ± 8
HOMA _{IR}	5.84 ± 3.58
Triglycerides, mg/dl	83 ± 48
Total cholesterol, mg/dl	167 ± 31
HDL cholesterol, mg/dl	47 ± 10
LDL cholesterol, mg/dl	103 ± 29
CRP, mg/dl	0.44 ± 0.36
C3, g/l	1.25 ± 0.2

All patients had reported to have normal fasting blood glucose (FBG) levels and, actually, all of them had a FBG < 110 mg/dl.

All study subjects were judged to be in good health on the basis of physical examination, medical history, routine blood work, urinalysis, and ECG.

Free thyroid hormones and thyroid-stimulating hormones (TSH) (<4.0 μUI/ml) were in the normal range in all subjects. None of the patients were receiving any kind of medication (including oral contraceptives for premenopausal women and hormone replacement therapy for postmenopausal women) when they entered the study. Moreover, none of them had been involved into intensive or competitive physical activity prior to the enrollment. During the testing period, all subjects were asked to keep their normal mixed diet and not to perform any sporting activity.

Measurements of the Common Carotid Intima-Media Thickness

Determinations of CCA-IMT were performed as previously described [18, 19]. Briefly, measurements were obtained from the far wall of the distal common carotid arteries (immediately proximal to the carotid bulb) and reported as the mean value for the bilateral measurement. This location was chosen a priori because of its demonstrated reproducibility, compared with measurements of CCA-IMT at other sites [19, 20].

All studies were performed on a single ultrasound machine (Hewlett Packard Sonos 1500B) using a linear-array 8.0 MHz scan head with standardized image settings, including resolution mode, depth of field, gain, and transmit focus. Ultrasound study was performed in a standard fashion by an examiner who was specifically trained to perform the prescribed study examination. All sonograms were obtained with the patient in the supine position and the head turned slightly to the contralateral side. Each ultrasound examination was performed as an independent study, without any knowledge of the family history of type 2 diabetes. The near-field (intimal-luminal surface) and far-field (medial-adventitial) arterial wall borders were manually traced for measurement of minimal and maximal CCA-IMT.

Anthropometric Measurements and General Data

Weight was measured to the nearest kg. Height was determined to the nearest cm. BMI was calculated as the weight (kg) divided by the square of height (m). Waist circumference was measured at the narrowest part of the abdomen, i.e. at the natural indentation between the 10th rib and the iliac crest (minimum waist).

Blood pressure was recorded on at least three different occasions, using a mercury manometer with an appropriate cuff size.

Table 2. Linear correlations between common carotid intima-media thickness and continuous variables (n = 140)

	r	p
Age, years	0.211	0.012
Body mass index, kg/m ²	0.482	<0.001
Waist circumference, cm	0.507	<0.001
Systolic blood pressure, mm Hg	0.277	0.001
Diastolic blood pressure, mm Hg	0.221	0.009
Fasting insulin, μUI/ml	0.331	<0.001
Fasting blood glucose, mg/dl	0.213	0.011
HOMA _{IR}	0.361	<0.001
Triglycerides, mg/dl	0.174	0.040
Total cholesterol, mg/dl	0.183	0.030
HDL cholesterol, mg/dl	-0.146	0.09
LDL cholesterol, mg/dl	0.188	0.026
CRP, mg/dl	0.311	<0.001
C3, g/l	0.357	<0.001

Hormonal and Metabolic Parameters

Blood samples were drawn between 08:00 and 09:00 a.m. after an overnight fast.

Serum insulin concentrations were measured by radioimmunoassay (Behring, Scoppito, Italy), and intra- and interassay coefficients of variation were 3.7 and 7.5%, respectively.

The C3 fraction of the complement was determined by immunonephelometry [21] (Behring Nephelometer II (BNII); Dade-Behring, Marburg, Germany). Intra-assay coefficient of variation was lower than 5.5%.

CRP was measured by a particle-enhanced turbidimetric immunoassay (PETIA) technique (Siemens Healthcare Diagnostic Inc., Newark, DE, USA). Intra- and interassay coefficients of variation were lower than 8%. It is noteworthy that this assay meets the recommendations of the American Heart Association and the Centers for Disease Control (2003) for determining patients at high risk for cardiovascular disease, but it cannot be used to differentiate low from average risk [22].

Plasma glucose levels were determined by the glucose-oxidase method (Sclavo, Siena, Italy). Plasma lipids (triglycerides, total cholesterol, and high-density lipoprotein (HDL) cholesterol) were determined by an automatic colorimetric method (Hitachi; Boehringer Mannheim, Mannheim, Germany).

Insulin resistance was assessed by using HOMA_{IR} [23].

Statistics

Results are presented as mean and standard deviation (SD) for all parameters. Significant relationships between study parameters were evaluated by Pearson's correlation coefficient. A multiple linear regression analysis was also performed to test the joint effect of different variables on CCA-IMT and C3. To avoid multicollinearity, variables with reciprocal Pearson's correlation coefficient <0.90 were introduced in the multivariate model. All statistical analyses were performed using the STATISTICA 6.0 for Windows (StatSoft Inc.; Tulsa, OK, USA).

Results

Table 1 shows the general, anthropometric, hormonal and metabolic parameters of subjects under study.

Table 2 shows the univariate relationship between CCA-IMT and all the continuous parameters investigated in this study. CCA-IMT was significantly and positively correlated

with all the parameters, except for HDL cholesterol. The strongest statistical association ($p < 0.001$) was with BMI, waist circumference, systolic blood pressure, insulin resistance (HOMA_{IR}), and insulin, CRP, and C3 serum levels.

Table 3 shows the relationship between C3 concentrations and all the continuous parameters investigated in this study. C3 levels were significantly correlated with all the parameters, except for age and fasting blood glucose. The strongest statistical association ($p < 0.001$) was with BMI, waist circumference, insulin resistance (HOMA_{IR}), and insulin, CRP, and triglyceride serum levels.

Table 4 shows the results of the multiple linear regression analysis of variables in relation to CCA-IMT ($R^2 = 0.37$; $p < 0.001$) and C3 ($R^2 = 0.509$; $p < 0.001$). To avoid multicollinearity, only variables with reciprocal Pearson's correlation coefficient < 0.90 were introduced in the multivariate model. Therefore, BMI was excluded from the model because of its high correlation with waist circumference ($r = 0.90$), total cholesterol was excluded because of its high correlation with low-

density lipoprotein (LDL) cholesterol ($r = 0.94$), and fasting insulin was excluded because of its high correlation with HOMA_{IR} ($r = 0.98$). Male gender and waist circumference maintained an independent positive relationship with the CCA-IMT, while waist circumference, triglyceride blood levels, and CRP maintained a positive and independent association with C3.

Discussion

The present study, performed in a population of healthy euthyroid subjects, mainly overweight and obese, shows a positive relationship between C3 serum levels and the CCA-IMT. These findings are apparently in line with the information that C3 is present in the atherosclerotic plaque [2] and that it is a strong predictor of myocardial infarction [3].

It is well known that abdominal obesity is independently related to the thickening of the CCA-IMT [11–17], and our data could suggest that one of the pathways by which central fat accumulation could bring about an atherosclerotic process would be by means of an immunologic reaction, in which C3 may be a very important factor. This hypothesis seems to be possible, since C3 was strongly associated with BMI and waist circumference in our subjects. The multiple linear regression pointed out an association between C3 and CRP, a well-known marker of inflammation related to the cardiovascular risk profile of patients. Moreover, it is well known that adipocytes express $\text{TNF-}\alpha$ and IL-6 [9], and this hyperproduction of cytokines would induce a condition of low-grade chronic inflammation that would be responsible for the activation of the complement system [10]. In addition, adipocytes are per se an important source of C3 production [4], and the gene expressions that code the proteins activating the complement system have been verified in human adipocytes [5].

However, the association between C3 levels and IMT was not maintained after adjustment of data for age, male gender,

Table 3. Linear correlations between C3 levels (g/l) and continuous variables ($n = 140$)

	r	p
Age, years	0.051	0.55
Body mass index, kg/m^2	0.592	< 0.001
Waist circumference, cm	0.634	< 0.001
Systolic blood pressure, mm Hg	0.185	0.028
Diastolic blood pressure, mm Hg	0.176	0.037
Fasting insulin, $\mu\text{UI}/\text{ml}$	0.427	< 0.001
Fasting blood glucose, mg/dl	−0.025	0.76
HOMA_{IR}	0.402	< 0.001
Triglycerides, mg/dl	0.362	< 0.001
Total cholesterol, mg/ml	0.208	0.014
HDL cholesterol, mg/dl	−0.232	0.006
LDL cholesterol, mg/dl	0.183	0.030
CRP, mg/dl	0.507	< 0.001

Table 4. Multiple linear regression of CCA-IMT and C3

	IMT ($R^2 = 0.365$; $p < 0.001$)		C3 ($R^2 = 0.509$; $p < 0.001$)	
	$\beta \pm \text{SE}$	p	$\beta \pm \text{SE}$	p
Age	0.0038 ± 0.0021	0.07	-0.0037 ± 0.0038	0.34
Male gender	0.0507 ± 0.0183	0.006	-0.0425 ± 0.0340	0.21
Waist circumference	0.0021 ± 0.0008	0.007	0.0063 ± 0.0013	< 0.001
Systolic blood pressure	-0.0003 ± 0.0008	0.69	-0.0013 ± 0.0015	0.40
Diastolic blood pressure	0.0008 ± 0.0012	0.48	0.0012 ± 0.0022	0.58
Hypertension	0.0230 ± 0.0275	0.40	-0.0510 ± 0.0500	0.31
Fasting blood glucose	0.0013 ± 0.0009	0.18	-0.0030 ± 0.0017	0.08
HOMA_{IR}	0.0033 ± 0.0025	0.19	0.0047 ± 0.0046	0.31
Triglycerides	-0.0001 ± 0.0002	0.42	0.0008 ± 0.0003	0.008
HDL cholesterol	0.0006 ± 0.0008	0.50	0.0006 ± 0.0015	0.70
LDL cholesterol	0.00002 ± 0.0003	0.93	0.0005 ± 0.0005	0.26
CRP	-0.0074 ± 0.0260	0.78	0.1042 ± 0.0465	0.027
C3	0.0577 ± 0.0487	0.24	–	–
IMT	–	–	0.1912 ± 0.1613	0.24

waist circumference, and factors associated with abdominal obesity, such as the level of insulin resistance, systolic blood pressure, and CRP, triglyceride, and LDL cholesterol levels. Therefore, this study seems to exclude that an immunologic reaction is one of the pathways by which central obesity could accelerate the atherosclerotic process.

The study confirms that male gender accelerates the atherosclerotic process and that C3 levels are associated with insulin levels [6]. In particular, it has been shown that serum C3 is an even stronger inflammatory marker of insulin resistance than CRP, leukocyte count, and erythrocyte sedimentation rate [7].

This study also shows a strong correlation of C3 levels with CRP and triglyceride circulating levels. The association with CRP levels had been previously reported in patients affected by type 2 diabetes [24], whereas the relationship with triglyceride is a further confirmation of previous studies [25].

Besides, the small sample adopted in our research may be an apparent limitation of the study. Apart from the adopted criteria, this is due to the involvement of only one centre in this study.

In conclusion, the present study shows that central fat accumulation, male gender, and age are the most important factors that independently increase the thickness of the arterial wall. Moreover, it shows the lack of an independent association between inflammation and inflammatory markers and the CCA-IMT, despite their association with central weight gain, thus excluding that an immunologic reaction is one of the pathways by which central obesity could accelerate the atherosclerotic process.

Disclosure Statement

The authors declare no conflict of interest.

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