Serum C3 Is a Stronger Inflammatory Marker of Insulin Resistance Than C-Reactive Protein, Leukocyte Count, and Erythrocyte Sedimentation Rate

Comparison study in an elderly population

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OBJECTIVE — This study was performed to ascertain the relative relevance of some inflammatory markers in insulin resistance.

RESEARCH DESIGN AND METHODS — Four inflammatory markers (leukocyte count, erythrocyte sedimentation rate [ESR], high-sensitivity C-reactive protein [CRP], and C3 complement) were assessed as possible determinants of the homeostasis model assessment (HOMA) index, together with the five elements of the metabolic syndrome (National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults [Adult Treatment Panel III] definition), total cholesterol, physical activity, and four indicators of adiposity (BMI, waist circumference, percent body fat, and hepatic steatosis) in an unselected population of 990 subjects aged 65–91 years (the Pianoro Study).

RESULTS — In univariable analysis, C3, CRP, and leukocyte count, but not ESR, were significantly correlated with HOMA index. In multivariable analysis, C3 remained associated with insulin resistance with the highest partial R^2 value (0.049), independently of all other covariates. The other most significant ($P < 0.0001$) determinants of HOMA index were total cholesterol (inverse association, $R^2 = 0.026$), waist circumference ($R^2 = 0.023$), triglycerides ($R^2 = 0.022$), and hepatic steatosis ($R^2 = 0.021$) ($R^2 = 0.450$ for the whole model).

The adjusted relative risks of having the metabolic syndrome for the subjects with inflammatory markers in the high tertile, with respect to those with lower values, were (prevalence ratio [95% CI]): 1.77 (1.41–2.22) for C3, 1.38 (1.12–1.70) for leukocyte count, 1.17 (0.94–1.46) for CRP, and 1.13 (0.91–1.40) for ESR.

CONCLUSIONS — Of the four inflammatory markers simultaneously assessed in our elderly population, only C3 was strongly associated with insulin resistance, independently of the components of the metabolic syndrome and the main indexes of abdominal and general obesity.

Diabetes Care 30:2362–2368, 2007

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Received for publication 2 April 2007 and accepted in revised form 11 June 2007.

Published ahead of print at http://care.diabetesjournals.org on 26 June 2007. DOI: 10.2337/dc07-0637.

Additional information for this article can be found in an online appendix at http://dx.doi.org/10.2337/dc07-0637.

Abbreviations: CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; HOMA, homeostasis model assessment.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Both insulin resistance and the metabolic syndrome are usually associated with an increase in inflammatory markers. In this respect, C-reactive protein (CRP) has been the most studied marker (1–6), but many other variables (7,8), including leukocyte count (9–12), erythrocyte sedimentation rate (ESR) (10,13), and C3 complement (14–18), have been assessed in relation to both conditions. Although a definite explanation for this phenomenon has not yet been found, one possibility is that the inflammatory cytokines that stimulate the hepatic production of acute-phase proteins are mainly secreted by the adipose tissue in excess (19,20) and that such cytokines may favor the appearance of insulin resistance by indirectly causing the phosphorylation and proteosomal degradation of insulin receptor substrates or by indirectly interfering with the insulin receptor substrate interaction (21).

Since many markers of inflammation have been associated with insulin resistance, metabolic syndrome, and diabetes, it is unclear whether any of them play a preeminent role (partially reflected in other correlated markers), and very few comparison studies have been performed so far. Thus, the present study was performed to ascertain which of four markers of inflammation (leukocyte count, ESR, CRP, and C3) was mostly associated with insulin resistance (as defined according to the homeostasis model assessment [HOMA] [22]) in a wide population of elderly subjects of both sexes. In addition, the need to adjust the analysis for possible confounders has also provided the opportunity to reexamine other insulin resistance–associated variables.

RESEARCH DESIGN AND METHODS

The Pianoro Study

The Pianoro Study is a wide epidemiological investigation on the lifestyle and the effects of physical activity in an Italian el-

derly population. The study started in November 2003, when a postal questionnaire was sent to all the subjects aged >65 years of both sexes (3,255 people) who were residents of the Pianoro municipality (northern Italy). The questionnaire was returned by 2,022 subjects (62%). Through the questionnaire, information was obtained concerning risk factors for atherosclerosis, lifestyle, previous cardiovascular diseases, quality of life, degree of autonomy, medications prescribed, and physical activity during the last week (Physical Activity Scale for the Elderly, including a section for household activities and a section for leisure-time activities) (23).

In the subsequent phase, the same subjects were invited for an assessment at their general practitioner's office to sign the informed consent; to check the questionnaire; to measure weight, height, and blood pressure; to verify previous ischemic events; and to perform a cognitive test. Finally, 7–10 days later, a fasting blood sampling; a general physical examination, including anthropometry; an electrocardiogram; an abdominal ultrasound assessment; and a submaximal step test were performed at the S.Orsola-Malpighi Hospital in Bologna. There were 1,163 participants in this second phase (36% of those initially invited).

All the subjects without any missing data were included in the present study, except the four insulin-treated subjects who were excluded. Thus, our population consists of 990 subjects, 485 men and 505 women, aged 65–91 years. The study was approved by the joint university-hospital ethical committee.

Table 1—*Characteristics of the study population*

Variable	Value	Range
Age (years)	72.6 ± 5.4	65–91
Male sex	485 (49.0)	—
Current smoker	90 (9.1)	—
Alcohol drinker	561 (56.7)	—
PASE score	8.6 (0.5–18.9)	0–100
Hypertension	671 (67.8)	—
Cholesterol (mmol/l)	5.61 ± 0.97	2.69–12.80
Triglycerides (mmol/l)	1.16 (0.89–1.60)	0.41–8.74
HDL cholesterol (mmol/l)	1.54 ± 0.38	0.59–3.10
Creatinine (μmol/l)	84.0 (72.5–96.4)	38.9–314.7
Hematocrit	0.41 ± 0.04	0.26–0.55
Previous myocardial infarction	55 (5.5)	—
Previous stroke	27 (2.7)	—
Blood glucose (mmol/l)	5.44 (4.99–5.99)	3.55–16.04
Insulin (mU/l)	8.0 (5.6–11.9)	1.2–60.3
HOMA index	1.9 (1.3–3.1)	0.3–22.0
Diabetes	143 (14.4)	—
Impaired fasting glucose	307 (31.0)	—
Metabolic syndrome	266 (26.9)	—
BMI (kg/m^2)	26.4 ± 4.0	16.4–43.1
Waist circumference (cm)	96.2 ± 11.0	60–150
Percent body fat	34.2 ± 7.4	7.8–49.9
Hepatic steatosis	433 (43.7)	—
Leukocyte count (n 10^{-9}/l)	5.92 (4.97–6.96)	2.75–20.77
ESR (mm/h)	17 (11–30)	1–112
C3 complement (g/l)*	1.29 ± 0.21	0.65–2.12
CRP (mg/dl)	0.19 (0.10–0.39)	0.01–19.71

Data are means ± SD, median (interquartile range), or n (%). *Median (interquartile range) for C3 was 1.27 g/l (1.14–1.41). PASE, Physical Activity Scale for the Elderly (leisure-time score).

Variables examined

BMI is the ratio between weight in kilograms and the square of height in meters. Waist circumference was measured with the patient standing, at the umbilicus level. Body fat, expressed as percentage of weight, was estimated on the basis of the measurement of four skinfolds (bicipital, tricipital, subscapular, and suprailiac) (24). Alcohol consumption was defined as the consumption of at least one alcoholic unit (a glass of wine or a pint of beer or a small glass of spirit) per day. The subjects who were treated with antidiabetes drugs and/or who had fasting glucose levels ≥7.0 mmol/l (126 mg/dl) were considered diabetic, while those with glucose levels ≥5.55 mmol/l (100 mg/dl) who were not diabetic were considered to have impaired fasting glucose. The subjects with a history of hypertension and/or systolic blood pressure ≥160 mmHg and/or diastolic pressure ≥95 mmHg (measurement performed with the patient sitting, at the end of the first visit) were considered hypertensive. These pressure limits were preferred to the more usual 140/90 mmHg due to the very high prevalence of hypertensive subjects in this population (see RESULTS).

Hepatic steatosis was classified as absent, mild, moderate, or severe according to the ultrasound criteria of Joseph et al. (25). In the present study, hepatic steatosis was defined as the presence of at least a mild pattern (a hyperechoic liver with fine, tightly packed echoes). The following hematochemical measurements were performed on the same day of blood sampling: complete blood count, ESR, C3 complement, blood glucose, creatinine, total cholesterol, HDL cholesterol, and triglycerides. Insulin and high-sensitivity CRP were measured a few months later in aliquots of serum frozen at −80°C.

The leukocyte count was measured automatically by a Bayer ADVIA 120 counter. The ESR was also measured automatically by the stopped-flow technique in a capillary microphotometer (Alifax Test 1 System). Serum C3 and high-sensitivity CRP were measured by immunoturbidimetric methods with commercially available kits (Roche Diagnostics, Mannheim, Germany) Tina-quant C3c and Tina-quant CRP (Latex) HS, respectively. Their analytical sensitivity was 0.04 g/l for C3 and 0.01 mg/dl for CRP. Insulin was measured by an electrochemiluminescence immunoassay (Insulin Elecsys; Roche Diagnostics), with an analytical sensitivity of 0.2 mU/l. Intra- and interassay coefficients of variation were, respectively, 1.4 and 2.6% for C3, 0.4 and 2.4% for CRP, and 1.2 and 4.3 for insulin. Insulin resistance was estimated with the HOMA index (22): (insulin [mU/l] × blood glucose [mmol/l])/22.5.

The presence of metabolic syndrome was established according to the definition of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment

Table 2—*Univariable associations with insulin resistance, according to HOMA index tertiles*

	HOMA index tertiles					
	Low (<1.52)	Middle (1.52–2.64)	High (≥2.65)	*F*	χ^2	*P* value
n	327	334	329			
Age (years)	73.0 ± 5.8	72.5 ± 5.3	72.4 ± 5.1	1.4	—	0.26
Male sex	153 (46.8)	157 (47.0)	175 (53.2)	—	3.5	0.18
Triglycerides (mmol/l)	1.01 (0.79–1.38)	1.12 (0.91–1.49)	1.48 (1.05–2.06)	59.9	—	<0.0001
Cholesterol (mmol/l)	5.73 ± 0.89	5.67 ± 1.05	5.45 ± 0.95	7.6	—	0.0005
HDL cholesterol (mmol/l)	1.70 ± 0.39	1.55 ± 0.34	1.37 ± 0.33	68.5	—	<0.0001
Hypertension	186 (56.9)	220 (65.9)	265 (80.5)	—	44.2	<0.0001
PASE score (high tertile)	124 (37.9)	107 (32.0)	91 (27.7)	—	7.9	0.02
BMI (kg/m^2)	24.2 ± 3.3	26.4 ± 3.4	28.7 ± 3.9	130.0	—	<0.0001
Waist circumference (cm)	90.0 ± 9.9	96.2 ± 9.4	102.4 ± 10.0	133.4	—	<0.0001
Percent body fat	31.6 ± 7.4	34.5 ± 7.2	36.3 ± 6.8	34.9	—	<0.0001
Hepatic steatosis	74 (22.6)	129 (38.6)	230 (69.9)	—	153.7	<0.0001
Metabolic syndrome	25 (7.6)	64 (19.2)	177 (53.8)	—	193.0	<0.0001
CRP (mg/dl)	0.15 (0.07–0.29)	0.20 (0.11–0.39)	0.22 (0.12–0.47)	12.2	—	<0.0001
Leukocyte count (n 10^{-9}/l)	5.52 (4.70–6.63)	5.95 (4.94–6.87)	6.32 (5.45–7.12)	18.9	—	<0.0001
C3 complement (g/l)*	1.21 ± 0.19	1.28 ± 0.19	1.37 ± 0.22	58.9	—	<0.0001
ESR (mm/h)	16 (11–28)	17 (11–30)	18 (11–31)	0.7	—	0.50

Data are means ± SD, median (interquartile range), or *n* (%). *Median (interquartile range) for C3 was 1.21 g/l (1.08–1.33), 1.28 g/l (1.15–1.39), and 1.37 g/l (1.22–1.49) for low, middle, and high index tertiles, respectively. PASE, Physical Activity Scale for the Elderly (leisure-time score).

Panel III) (26), which states that at least three of the following five criteria must be satisfied: *1*) waist circumference >102 cm in men and >88 cm in women, *2*) blood glucose ≥110 mg/dl (6.1 mmol/l), *3*) triglycerides ≥150 mg/dl (1.7 mmol/l), *4*) HDL cholesterol <40 mg/dl (1.0 mmol/l) in men and <50 mg/dl (1.3 mmol/l) in women, and 5) systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥85 mmHg.

Statistical analysis

The statistical analysis was mainly performed by parametric tests. Thus, several variables with asymmetric distribution to the left were logarithmically transformed (base e) in order to obtain substantially normal distributions before performing the tests. The ln-transformed variables were HOMA index, insulin, blood glucose, triglycerides, creatinine, CRP, leukocyte count, and ESR. The Physical Activity Scale for the Elderly score could not be normalized, and, when necessary, it was categorized (high tertile = 1, low + middle tertile = 0). To describe these variables, median (interquartile range) were used, while means ± SD were used for the normally distributed variables.

The differences among means in HOMA tertiles were assessed by ANOVA. The χ^2 test was used for comparisons among percentages. Linear regression analysis was performed to exclude any significant collinearity among inflammatory markers and among obesity indexes before performing multivariable analysis. The independent determinants of the logarithm of HOMA index were assessed by four models of multiple linear regression of increasing complexity. Partial R^2 was used to describe the strength of the association of each variable, tolerance values were examined to exclude any collinearity within the models, and an overall R^2 was also computed for each model. The adjusted relative risks of having the metabolic syndrome or hepatic steatosis, associated with the high tertile of the inflammatory markers, were assessed by Cox regression with assignment of a constant risk period to every subject and robust variance estimate, which allowed the calculation of correct prevalence ratios and 95% CIs (27). Two-tailed tests were used throughout, considering significant *P* values <0.05. The statistical analysis was carried out with Stata statistical software (version 9.0).

RESULTS

Characteristics of the study population

On average, the population was overweight (mean BMI 26.4 kg/m^2), two-thirds of the subjects were hypertensive, the majority (57%) drank at least one alcoholic unit per day, 44% presented ultrasonographic signs of hepatic steatosis, 31% had impaired fasting glucose, 27% satisfied the criteria for the diagnosis of metabolic syndrome, and nearly 14% were diabetic (Table 1). As far as the medications were concerned, 43.4% of the subjects were taking ACE inhibitors/angiotensin II receptor blockers, 20.3% lipid-lowering drugs, 20.2% β-blockers, 18.9% antiplatelet drugs, 9.8% nonsteroidal anti-inflammatory drugs, 8.9% diuretics, 7.0% oral antidiabetes drugs, and 2.2% corticosteroids.

The 1,032 subjects who returned the postal questionnaire, but did not participate in the present investigation, were not different from the study population as far as the prevalence of diabetes, hypertension, alcohol consumption, and current smoking were concerned. However, they differed significantly in many respects from the 990 participants: they were older (75.8 ± 7.6 years), less often male (43.4%), less overweight (BMI 25.8 kg/m^2), less educated (26.9 vs. 33.4% with ≥5 years of school activity), and had a lower prevalence of hypercholesterolemia (25.1 vs. 34.6%). On the other hand, they had an almost double prevalence of previous myocardial infarction (9.1%) and stroke (5.4%).

Determinants of insulin resistance

The nonadjusted behavior in relation to HOMA index tertiles of the main variables related to insulin resistance, as well as of the four markers of inflammation considered in this study, is shown in Table 2. In

Table 3—*Multivariable associations with ln(HOMA index) in four linear regression models of increasing complexity*

	1		2		3		4	
Covariate	R^2	*P* value	R^2	*P* value	R^2	*P* value	R^2	*P* value
ln(triglycerides)	0.066	<0.0001	0.051	<0.0001	0.030	<0.0001	0.022	<0.0001
Cholesterol	0.021*	<0.0001	0.018*	<0.0001	0.024*	<0.0001	0.026*	<0.0001
HDL cholesterol	0.023*	<0.0001	0.005*	0.03	0.006*	0.02	0.007*	0.01
Hypertension	0.023	<0.0001	0.013	0.0003	0.012	0.0007	0.007	0.007
PASE score (high tertile)	0.006*	0.01	0.004*	0.04	0.005*	0.03	0.003*	0.07
Waist circumference			0.167	<0.0001	0.158	<0.0001	0.023	<0.0001
BMI							0.006	0.01
Percent body fat							0.009	0.004
Hepatic steatosis							0.021	<0.0001
ln(CRP)	0.010	0.002	0.001	0.37	0.010*	0.002	0.010*	0.001
ln(leukocyte count)							0.005	0.02
C3 complement					0.081	<0.0001	0.049	<0.0001
Model	0.244	<0.0001	0.370	<0.0001	0.421	<0.0001	0.450	<0.0001

*Inverse relationship. The R^2 values shown are partial coefficients. PASE, Physical Activity Scale for the Elderly (leisure-time score).

our population, age and sex were not significantly associated with insulin resistance. Total cholesterol, HDL cholesterol, and physical activity were instead inversely related to HOMA index, while all the obesity indicators, triglycerides, and the prevalence of hypertension and of hepatic steatosis were directly associated with insulin resistance. Also, the inflammatory markers were directly associated with HOMA index, except ESR, whose relationship was not significant (online appendix Fig. 1 for regression lines [available at http://dx.doi.org/10.2337/dc07-0637]). Among them, the strongest association concerned C3 ($F = 58.9$, $P < 0.0001$). Waist circumference was the single continuous variable most strongly associated with insulin resistance.

Before performing the multivariable analysis, the correlation matrices among inflammatory markers and obesity indexes were examined. C3 and CRP were the most correlated inflammatory markers ($r = 0.47$, $P < 0.0001$), while leukocyte count and ESR were the least correlated ($r = 0.11$, $P = 0.0008$). Among obesity indexes, waist circumference and BMI were the most correlated ($r = 0.84$, $P < 0.0001$), while waist circumference and percent body fat were the least correlated ($r = 0.30$, $P < 0.0001$).

Table 3 reports the multivariable associations of the logarithm of HOMA index with all the variables significantly associated with it in univariable analysis. Four models of increasing complexity are illustrated. The first model describes the associations with serum lipids, hypertension, leisure-time physical activity, plus CRP (a marker of inflammation), which in this model was associated with insulin resistance independently of the other variables. In model 2, an obesity index (waist circumference) was added to the previous variables. Waist circumference had the strongest association, while CRP was no longer significantly associated with ln(HOMA). In the third model, C3 was also added. This variable was the second, after waist circumference, among the variables most strongly associated with insulin resistance. CRP regained a significant association with insulin resistance but of the inverse type. Finally, model 4 illustrates the effect of adding leukocyte count plus three further indexes of general and abdominal obesity (BMI, percent body fat, and hepatic steatosis). All these indexes remained independently associated, together with waist circumference, with ln(HOMA). In this model, C3 had the strongest relationship ($R^2 = 0.049$) and was followed by cholesterol (inverse relationship, $R^2 = 0.026$), waist circumference ($R^2 = 0.023$), triglycerides ($R^2 = 0.022$), and hepatic steatosis ($R^2 = 0.021$). Leukocyte count was weakly associated with insulin resistance, while CRP retained its inverse association. This model was able to explain 45% of HOMA index variability. Results did not change substantially, controlling also for age, sex, and medication with antidiabetes drugs, lipid-lowering drugs, anti-inflammatory nonsteroidal and steroidal drugs, β-blockers, and diuretics. Since many independent variables were interrelated, the possible presence of collinearity (and consequent analysis distortion) was carefully checked, considering the tolerance value of each variable within each model. The minimum tolerance value (0.26) was reached by BMI within model 4. This value is well over the limit of 0.1, below which a collinearity problem usually arises.

A model including ln(HOMA) as a dependent variable and waist circumference, triglycerides, HDL cholesterol, and hypertension as independent variables (i.e., the elements of the metabolic syndrome according to the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults [Adult Treatment Panel III] criteria [26] without blood glucose, as this was comprised in the HOMA formula) reached an overall R^2 of 0.354. A similar model, in which the independent variables were waist circumference, C3, triglycerides, and total cholesterol (i.e., the variables most strongly associated with HOMA index in model 3) reached an R^2 of 0.400.

Finally, the relative risk of having metabolic syndrome for the subjects with inflammatory markers in the high tertile, with respect to the subjects with lower values, was calculated within a Cox regression model with a robust variance estimate that included the four inflammatory markers (low and middle tertile = 0; high tertile = 1), plus age, sex, and medication (see above) as independent variables. According to this analysis, the relative risks (prevalence ratio [95% CI]) were 1.77 [1.41–2.22], $P < 0.0001$ for C3 levels ≥1.37 g/l; 1.38 [1.12–1.70], $P = 0.002$ for a leukocyte count ≥6.6×10^9/l; 1.17 [0.94–1.46], $P = 0.16$ for CRP levels ≥0.30 mg/dl; and 1.13 [0.91–1.40], $P = 0.26$ for an ESR ≥26 mm/h. A

similar assessment to estimate the adjusted prevalence ratios for hepatic steatosis provided the following results: 1.55 [1.33–1.80], $P < 0.0001$ for C3 levels ≥1.37 g/l; 1.16 [1.00–1.33], $P = 0.048$ for a leukocyte count ≥6.6×10^9/l; 1.04 [0.89–1.22], $P = 0.59$ for CRP levels ≥0.30 mg/dl; and 0.94 [0.80–1.10], $P = 0.43$ for an ESR ≥26 mm/h.

CONCLUSIONS

Preeminent role of C3 complement in insulin resistance

This study has compared for the first time C3 complement, CRP, ESR, and leukocyte count as determinants of the HOMA index in a wide elderly population. Our analysis has shown that when these inflammatory markers were simultaneously assessed together with the main conventional determinants of insulin resistance, serum C3 was by far the inflammatory marker most strongly correlated with HOMA index. Indeed, C3 was the second strongest covariate of HOMA index after obesity, when the latter was synthetically represented by waist circumference, and even became the first covariate when obesity was represented by four variables (BMI, percent body fat, waist circumference, and hepatic steatosis).

The strong association of C3 with insulin action and fasting insulin has already been reported in young adult Pima Indians (15) and in men aged 55–64 years (14). More recently, Engström et al. (17) have demonstrated that C3 is a powerful risk factor for the development of diabetes in men aged 38–50 years, even after adjustment for fibrinogen, haptoglobin, orosomucoid, α1-antitripsin, ceruloplasmin, and C4. Since this study did not assess the most common inflammatory markers (i.e., CRP, ESR, and leukocyte count), we were prompted to compare C3 with such variables as inflammatory indicators of insulin resistance.

Besides being produced in the liver like other acute-phase proteins (28), C3 is also synthesized by activated macrophages (29) and adipocytes (30), therefore behaving as an inflammatory cytokine and an adipokine. Its hepatic production is induced by "primary wave" cytokines, such as interleukin-1 and tumor necrosis factor α (31), which may interfere with insulin receptor functioning and cause insulin resistance (21). Due to postmenopausal increase, after the age of 50 years C3 is higher in women than in men (as was the case in our population; data not shown), while in men levels tend to be substantially independent of age (32). Although serum C3 is associated with the main endogenous cardiovascular risk factors (14,16,32), it has been found to be strongly predictive of myocardial infarction independently of them (33). In addition, our study has shown that C3 is associated with insulin resistance independently of the main indexes of obesity. Evidently, and differently from CRP (see further), the association of C3 with insulin resistance is not mainly mediated by the adipose tissue.

Finally, the possibility exists that the increase in C3 levels may also be due to a mechanism that parallels insulin resistance but does not coincide with it. The main activation fragment of C3, C3a desArg (also known as acylation stimulating protein), is provided with insulin-like properties: it favors glucose transmembrane transport and the synthesis of triglycerides in adipocytes (34). Thus, similarly to the increase in insulin levels in the presence of insulin resistance, a hypothetical acylation stimulating protein resistance (35) might induce an increase in the levels of acylation stimulating protein precursor (i.e., C3).

Other inflammatory markers

Of the markers of inflammation associated with insulin resistance and metabolic syndrome, CRP has been the one so far most studied (1–6) and has even been proposed as a new criterion for the metabolic syndrome (36). However, in our population of elderly subjects, after adjustment for serum lipids, hypertension, and physical activity, the association of CRP with insulin resistance was not very strong (Table 3, *model 1*) and completely disappeared when waist circumference was added to the model. In substantial agreement with these results, Wannamethee et al. (5) found that after adjustment for waist circumference the relationship between CRP and HOMA was greatly weakened. CRP is strongly associated with adipose tissue and obesity (1,6), probably because its hepatic synthesis is stimulated by the interleukin-6 (37) produced in the adipose tissue (20). Thus, CRP does not seem to provide a significant independent contribution to insulin resistance but rather mainly reflects the important contribution of obesity. Indeed, after addition of C3 to the multivariable model, a significant inverse association of CRP with HOMA index appeared, so that CRP might even be considered a marker of insulin sensitivity (rather than insulin resistance) for reasons that are currently unknown. Finally, we found that CRP was not independently associated with the metabolic syndrome in a Cox regression model also including C3 and leukocytes.

Findings concerning noninflammatory parameters

Although interrelated, all four obesity parameters considered in this study were independently associated with insulin resistance. In this context, the rather strong contribution of hepatic steatosis, already proposed as a new component of the metabolic syndrome (38), deserves particular consideration.

In our population of elderly subjects, total cholesterol was strongly and inversely associated with insulin resistance. It has previously been reported that diabetic subjects in different ethnic groups (39,40) had lower levels of LDL cholesterol than nondiabetic subjects. These data may be interpreted taking into account that insulin resistance reduces the intestinal absorption of cholesterol, in addition to increasing its synthesis (41,42). Although these two phenomena are usually balanced, it is possible that in elderly people, in the presence of insulin resistance, the hepatic synthesis of cholesterol may not adequately increase, with consequent prevalence of the decreased absorption.

Study limitations

HOMA index is a surrogate marker of insulin resistance. The latter would be more precisely measured by clamping techniques, which however are not feasible in large epidemiological studies. Due to the missed participation of many of the oldest subjects and of those in worst health conditions, we cannot apply our results to all the subjects aged >65 years. To avoid an unpredictable modification of the characteristics of the original sample, we chose not to exclude the subjects on antidiabetes, lipid-lowering, and anti-inflammatory treatment, nor those who had previous events in the past. However, results did not change when the multivariable analysis was controlled for medication. Moreover, it seems improbable that these factors may have influenced the central subject of the study (i.e., the relative relevance of four inflammatory markers in insulin resistance).

In conclusion, among the markers of inflammation assessed in our elderly pop-

ulation, C3 had the strongest association with insulin resistance after adjustment for obesity indexes, other components of the metabolic syndrome, and other inflammatory markers. Future studies will have to address the relative relevance of C3 in comparison with cytokines and adipokines as determinants of insulin resistance.

Acknowledgments— The study was supported by grants from Fondazione Cassa di Risparmio in Bologna and Regione Emilia Romagna-Piani per la Salute.

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