Metabolic Impact on Serum Levels of Complement Component 3 in Japanese Patients

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The aim of this study was to explore the association between the serum concentration of complement component 3 (C3) and a variety of metabolic parameters. The study involved 125 patients in our outpatient clinic. Anthropometric and clinical laboratory data were collected and statistical associations between the serum concentration of C3 and other parameters were evaluated in a cross-sectional as well as a prospective manner. A group of male patients with metabolic syndrome (Mets, $n=35$) were characterized by marked increase in serum concentrations of C3, body mass index (BMI), waist circumference, hemoglobin (Hb) A1c, insulin resistance (HOMA-IR), triglyceride, uric acid, urinary protein, and Hb. In a one-way analysis of variance of all subjects, the serum concentration of C3 was significantly elevated as the number of

items of complying with the Mets diagnostic criteria increased. In 60 of 125 patients who did not have diabetes and were given antilipogenetic medication, the serum concentration of C3 showed significant positive associations with serum levels of CH50, insulin, HOMA-IR, total cholesterol, hematocrit, LDL-c, C4, Hb, triglyceride, BMI, and albumin. In a prospective follow-up evaluation ($n=35$), there was a significant positive association between Δ C3 (the second concentration of serum C3 minus the first concentration of serum C3) and Δ HOMA-IR (the second concentration of HOMA-IR minus the first concentration of HOMA-IR). In conclusion, in Japanese patients, there is evidence implicating C3 concentration as a marker of Mets coinciding with insulin resistance. *J. Clin. Lab. Anal.* 24:113–118, 2010. © 2010 Wiley-Liss, Inc.

Key words: C3; C3a desArg; acylation stimulating protein (ASP); metabolic syndrome; insulin resistance

INTRODUCTION

The level of serum complement component 3 (C3) is an indicator in a variety of clinical settings, such as systemic lupus erythematosus (SLE), cryoglobulinemia, malignant rheumatoid arthritis, membranoproliferative glomerulonephritis, and acute glomerulonephritis. In addition, patients with protein-energy malnutrition show low concentrations of serum C3 and their nutritional rehabilitation results in the recovery of the levels of serum C3 to the normal range (1–3), indicating that sufficient nutrition has a positive impact on the serum concentration of C3.

C3 is synthesized predominantly by hepatocytes, but a wide variety of other tissue and cell types are also capable of synthesizing complement proteins (4–7). Extrahepatic secreted C3 accounts for 5% of circulating

C3 in humans (8). Adipose tissue is a major source of factor D (fD; adipsin), in addition to C3 and factor B (fB), and these proteins are major starter molecules of the alternative complement pathway, both in vitro and in vivo (9). Pomeroy et al. described the effect of excess body weight (BW) and caloric restrictions on serum concentration of C3 (10). They concluded that levels of serum C3 are determined in part by factors that influence BW, possibly owing to changes in production

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of C3 in adipose tissue. In recent years, investigators have identified associations between hyperinsulinemia, fasting plasma glucose (FPG), body mass index (BMI), and serum concentration of C3 (11–13). The effect of excess nutrition state on the serum concentration of C3 has received considerable attention. However, the prevalence of metabolic syndrome and/or obesity varies by race and gender among adults (14,15), and the metabolic factors that contribute to serum levels of C3 have to date not been clearly defined in the native Japanese population. In this study, we undertook clinical research on excessive production of C3, focusing on the metabolic syndrome and insulin resistance.

MATERIALS AND METHODS

This study included 125 patients monitored in the outpatient clinic of Juntendo University Hospital, Divisions of Nephrology and Hypertension. The study was conducted in accordance with the Declaration of Helsinki (1964) and approved by the Institutional Review Board in Juntendo University. Patient demographics are given in Table 1. There were 80 men and 45 women. The mean ($\pm$ SD) age was 59.6 ± 12.9 years. After overnight fasting, height and BW were recorded. Waist circumference (WC) was measured in centimeters, using the circumference at the level of the navel. BMI was calculated by dividing BW (kilograms) by height (2) (square meters). Complications included hypertension ($n = 108$), type 2 diabetes mellitus ($n = 29$), and hyperuricemia ($n = 21$). Patients who were treated with statins ($n = 29$), icosapentate ($n = 10$), cholestyramine ($n = 3$), bezafibrate ($n = 1$) and corticosteroids ($n = 4$) were included. Patients diagnosed with SLE, polycystic kidney disease, type 1 diabetes mellitus, Cushing's disease, and thyroidal disease were excluded. Most of the enrolled patients were taking antihypertensives (calcium channel blockers, beta-blockers, furosemide, angiotensin converting enzyme inhibitors, and/or angiotensin receptor 1 antagonists).

All blood samples were collected from the antecubital vein after overnight fasting. Biochemical analysis, including C3, C4, CH50, hemoglobin (Hb), hematocrit (Hct), FPG, HbA1c, total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-c), LDL-cholesterol (LDL-c), serum creatinine (Cr), uric acid (UA), albumin, high sensitivity c-reactive protein (hs-CRP), and urinary protein was undertaken at the central laboratory in the Juntendo University Hospital. Insulin resistance was assessed using the Homeostasis Model Assessment (HOMA-IR): fasting serum insulin (μ U/ml) $\times$ FPG (mg/dl)/405 (16). Estimated glomerular filtration rate (eGFR) was calculated using the formula established by the Japanese Society of Nephrology:

$eGFR \text{ (ml/min/1.73 m}^2\text{)} = 194 \times Cr^{-1.094} \times \text{age}^{-0.287}$ (if female, $\times 0.739$) (17).

The original diagnostic definition of metabolic syndrome in Japan established by the Study Group on Criteria for the Metabolic Syndrome (18,19) was used. The subjects had to satisfy the following criteria: waist circumference of 85+ cm for males and 90+ cm for females as an essential criteria, combined with two or more of the following criteria: (1) TG of 150 mg/dl and/or HDL-c of <40 mg/dl; (2) systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg; and (3) FPG ≥ 110 mg/dl.

Data are given as mean $\pm$ standard deviation of the mean. One-way or 2-factor analysis of variance (ANOVA), followed by pair-wise comparisons with Scheffé's *F* analysis or χ^2 test when total ANOVA indicated a significant difference, were used in the comparisons between grouped items, respectively. Spearman's test to assess simple correlations between two variables was applied, and $P < 0.05$ was considered to be statistically significant for all analyses.

RESULTS

The mean concentration of serum C3 was 110.0 ± 23.6 mg/dl (normal range, 69 to 128 mg/dl) and showed a normal distribution (Fig. 1). BMI was 25.1 ± 4.12 kg/m² and WC was 84.8 ± 12.5 cm. FPG, HbA1c, TC, TG, HDL-c, LDL-c, Cr, UA, albumin, Hb, and Hct were within the normal ranges. eGFR was 62.7 ± 29.4 ml/min/1.73 m². Urinary protein was 0.7 ± 1.2 g/g $\cdot$ creatinine.

Impact of Metabolic Syndrome on Serum Concentration of C3

Forty-four subjects met the Japanese criteria for Mets (Table 1) (18,19). Because the Mets positive group (Mets+) included more male patients (79.5%) than the Mets negative group (Mets-) (55.5%), Mets+ and Mets- were divided by sex and analyzed by 2-factor ANOVA. There was no difference in age, blood pressure, C4, CH50, FPG, TC, HDL-c, LDL-c, Cr, albumin, eGFR, Hct, and hs-CRP between subgroups. Male patients with Mets+ are characterized by marked increase of BMI, WC, C3, HbA1c, HOMA-IR, TG, UA, urinary protein, and Hb.

All subjects were classified by the number of Mets diagnostic criteria: non-criterion group, $n = 10$; 1 criterion group, $n = 23$; 2 criteria group, $n = 41$; 3 criteria group, $n = 32$; and 4 criteria group, $n = 19$. One to four criteria groups, excluding non-criterion group, exhibited an increasing tendency with the number of Mets criteria (Fig. 2; one-way ANOVA, $P < 0.01$). Scheffé's *F* analysis revealed that there was a

TABLE 1. Clinical Background of All Subjects and Those with Metabolic Syndrome

		Mets+ (n = 44)		Mets− (n = 81)			
	Total (n = 125)	Male (n = 35)	Female (n = 9)	Male (n = 45)	Female (n = 36)	ANOVA (P)	Scheffe's F analysis
Anthropometric variables							
Age (years)	59.6 ± 12.9	58.6 ± 12.9	59.6 ± 15.4	57.2 ± 13.5	63.7 ± 11.3	NS	
Systolic blood pressure (mmHg)	134.7 ± 15.8	140.7 ± 14.6	141.6 ± 12.8	130.3 ± 15.4	133.0 ± 16.3	NS	
Diastolic blood pressure (mmHg)	77.7 ± 11.3	82.0 ± 13.8	77.3 ± 15.6	75.8 ± 7.5	76.2 ± 11.0	NS	
BMI (kg/m ²)	25.1 ± 4.12	27.5 ± 2.7	28.1 ± 2.9	23.8 ± 4.6	23.8 ± 3.4	<0.01	<0.01 ^a , <0.01 ^b , <0.05 ^c , <0.05 ^d
Waist circumference (cm)	84.8 ± 12.5	94.8 ± 7.3	94.1 ± 4.7	82.5 ± 11.7	76.5 ± 10.5	<0.01	<0.01 ^a , <0.01 ^b , <0.05 ^c , <0.01 ^d
Biochemical variables							
C3 (mg/dl)	110.0 ± 23.6	119.7 ± 25.4	123.4 ± 29.0	101.8 ± 20.8	106.5 ± 18.6	<0.01	<0.01 ^a
C4 (mg/dl)	26.5 ± 6.9	27.7 ± 7.1	28.0 ± 3.4	24.6 ± 5.8	27.4 ± 8.3	NS	
CH50 (Unit/ml)	45.4 ± 7.5	46.3 ± 7.0	50.5 ± 9.5	43.7 ± 7.3	45.2 ± 6.7	NS	
FPO (mg/dl)	102.8 ± 29.2	112.3 ± 49.5	104.4 ± 8.7	99.4 ± 11.8	97.6 ± 19.7	NS	
HbA1c (%)	5.6 ± 0.8	6.0 ± 1.2	5.7 ± 0.5	5.2 ± 0.3	5.4 ± 0.5	<0.01	<0.01 ^a , <0.05 ^b
HOMA-R	2.63 ± 2.94	4.1 ± 4.4	2.7 ± 1.0	1.7 ± 1.7	2.2 ± 2.2	<0.01	<0.01 ^a
Total cholesterol (mg/dl)	211.6 ± 43.4	215.8 ± 48.0	233.7 ± 52.9	202.9 ± 43.3	211.5 ± 34.5	NS	
Triglyceride (mg/dl)	166.6 ± 127.8	256.9 ± 186.9	182.0 ± 76.1	119.9 ± 59.5	128.7 ± 72.6	<0.01	<0.01 ^a , <0.01 ^b
HDL cholesterol (mg/dl)	54.5 ± 15.8	49.1 ± 14.4	53.5 ± 20.0	57.1 ± 15.6	56.2 ± 15.7	NS	
LDL cholesterol (mg/dl)	121.3 ± 34.9	121.2 ± 36.7	140.4 ± 42.3	115.6 ± 34.4	123.5 ± 31.4	NS	
Serum creatinine (mg/dl)	1.5 ± 1.7	1.5 ± 1.6	1.8 ± 2.5	1.4 ± 1.6	1.5 ± 1.9	NS	
Uric acid (mg/dl)	6.4 ± 1.3	6.8 ± 0.9	6.7 ± 1.8	6.3 ± 1.1	5.7 ± 1.4	<0.01	<0.01 ^b
Albumin (mg/dl)	4.2 ± 0.5	4.2 ± 0.5	4.3 ± 0.4	4.2 ± 0.5	4.1 ± 0.6	NS	
Estimated GFR (ml/min/1.73 m ²)	62.7 ± 29.4	63.1 ± 31.2	61.9 ± 35.7	63.4 ± 25.7	61.8 ± 31.4	NS	
Urinary protein (g/g creatinine)	0.7 ± 1.2	1.1 ± 1.5	0.6 ± 0.8	0.5 ± 1.2	0.3 ± 0.5	<0.05	<0.05 ^b
Hemoglobin (mg/dl)	13.4 ± 2.0	13.9 ± 2.3	13.2 ± 1.3	13.8 ± 1.8	12.3 ± 1.8	<0.01	<0.01 ^c , <0.05 ^f
Hematocrit (%)	40.9 ± 5.8	42.1 ± 6.5	40.8 ± 3.6	41.9 ± 5.4	38.4 ± 5.5	NS	
hs-CRP (μg/dl)	187 ± 316	72 ± 161	645 ± 838	176 ± 168	347 ± 534	NS	
Complications						χ ² (p)	
Hypertension	107	34	9	34	30	<0.05	
Dyslipidemia	74	29	9	18	18	<0.01	
Diabetes mellitus	29	14	3	1	5	<0.05	
Hyperuricemia	21	6	3	10	2	NS	
Therapy (n)							
Statins	29	7	3	8	11	NS	
Icosapentate	10	5	1	2	2	NS	
Cholestyramine	2	2	0	0	0	NS	
Bezafibrate	1	0	0	0	1	NS	
Corticosteroids	4	1	1	2	0	NS	

Values expressed as percent or mean ± SD. Abbreviations: Mets, metabolic syndrome; M, Tvlale; F, Female; BMI, body mass index; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; HOMA-R, homeostasis model assessment ratio; HDL, high-density lipoprotein; LDL, low density lipoprotein; GFR, glomerular filtration rate.

^aMets+Male vs. Mets–Male.

^bMets+Male vs. Mets–Female.

^cMets+Female vs. Mets–Male.

^dMets+Female vs. Mets–Female.

^eMets+Male vs. Mets–Female.

^fMets–Male vs. Mets–Female.

significant difference between the 1 criterion and 3 criteria groups ($P = 0.034$), 1 criterion and 4 criteria groups ($P = 0.0026$), and 2 criteria and 4 criteria groups ($P = 0.0464$).

Since an earlier report indicated that simvastatin has a lowering effect on the serum concentration of C3, we excluded patients prescribed with statins (12). To

evaluate further the metabolic impact of serum C3, we also excluded patients treated with icosapentate, cholestyramine, bezafibrate, corticosteroids, and hypoglycemic agents because these drugs can have a major influence on lipid metabolism. Sixty subjects (17 Mets+, 43 Mets–) remained and the clinical data were reevaluated. The serum concentration of C3 showed

Fig. 1. Histogram: serum concentration of C3. The mean concentration of C3 was 110.0 ± 23.6 mg/dl and, when presented, showed a normal distribution. The normal range of serum concentration of C3 in our hospital is 69–128 mg/dl.

Fig. 2. Serum concentration of C3 in five groups classified by number of compliant metabolic syndrome diagnostic criteria. One-way ANOVA revealed that serum concentration of C3 became significantly higher, as the number of compliant metabolic syndrome diagnostic criteria increased. (*Scheffé's *F* analysis).

significant positive associations with CH50, insulin, HOMA-IR, TC, Htc, LDL-c, C4, Hb, TG, BMI, and albumin (Table 2).

Data were collected for 35 subjects free of dietary restrictions at two time points in the following 6 months. Changes in the serum concentrations of C3 and clinical variables over this time period were evaluated. Figure 3 shows the relationship between Δ C3 (calculated from the second concentration of serum C3 minus the first concentration of serum C3) and Δ HOMA-IR (calculated from the second concentration of HOMA-IR minus the first concentration of HOMA-IR). Plots of 24 subjects (69.9%) are located in the first quadrant or third quadrant and show significantly positive relationships ($r = 0.690$, $R^2 = 0.476$, $P < 0.05$). However, there were no statistical differences between serum concentration of C3 and other variables.

Fig. 3. Scatter plots of two-point follow-up difference in 35 subjects. Plots of 24 subjects (69.9%) were located in the first quadrant or third quadrant and showed a significant positive relationship ($r = 0.690$, $R^2 = 0.476$, $P < 0.05$). However, there were no statistically significant differences between serum concentration of C3 and other variables.

TABLE 2. Relationship of Serum Concentration of C3 and Clinical Variables Assessed by Linear Regression Models

	<i>R</i>	<i>P</i>		<i>R</i>	<i>P</i>
CH50 (Unit/ml)	0.722	<0.0001	Serum creatinine (mg/dl)	−0.366	0.0037
Insulin (μU/ml)	0.572	<0.0001	Estimated GFR (ml/min/1.73 m ²)	0.347	0.0062
HOMA-IR	0.566	<0.0001	HbA1c (%)	0.283	0.0284
Total cholesterol (mg/dl)	0.535	<0.0001	Waist circumference (cm)	0.281	0.034
Hematocrit (%)	0.514	<0.0001	Systolic blood pressure (mmHg)	0.257	NS
LDL cholesterol (mg/dl)	0.510	<0.0001	Diastolic blood pressure (mmHg)	0.222	NS
C4 (mg/dl)	0.499	<0.0001	FPG (mg/dl)	0.209	NS
Hemoglobin (mg/dl)	0.467	0.0001	Uric acid (mg/dl)	0.196	NS
Triglyceride (mg/dl)	0.433	0.0005	hs-CRP (μg/dl)	−0.173	NS
BMI (kg/m ²)	0.427	0.0007	HDL cholesterol (mg/dl)	−0.148	NS
Albumin (mg/dl)	0.403	0.0019	Urinary protein (g/g-creatinine)	−0.104	NS
Age (years)	−0.372	0.0032			

NS, not significant

DISCUSSION

Adipose tissues are metabolically active and secrete a variety of paracrine and autocrine factors, such as leptin, TNF- α , and IL-6, all of which are thought to be involved in the regulation of BW and/or glucose homeostasis. In recent years, C3 has attracted a lot of attention as an adipocytokine. Human adipocytes synthesize and secrete C3, fB, and adipsin (4–7), and C3 interacts extracellularly with adipsin and fB to form C3a, from which the C-terminal arginine is removed by carboxypeptidases, generating C3a desArg, also known as acylation-stimulating protein (ASP). ASP is a potent stimulator of glucose transport and triglyceride synthesis in adipocytes (8,20,21).

Although racial and gender differences have earlier been identified in insulin resistance (14,15), this is the first report which explores the association between serum concentration of C3 and Mets in Japan. The male patients with Mets+ showed a higher concentration of serum C3 than male and female patients with Mets–, but the serum concentration of C4 and titer of CH50 did not differ between four groups. As the number of Mets diagnostic criteria increased, so did the serum concentration of C3 increase. Serum concentration of C3 was apparently influenced by insulin resistance. To clarify the association between C3 production and insulin resistance, we excluded patients who were prescribed statins, icosapentate, cholestyramine, benzaifibrate, and/or corticosteroids, and who had diabetes. The results became clearer when the serum concentration of C3 showed strongly positive association with a marker of Mets, such as insulin, HOMA-IR, TC, LDL, TG, and BMI.

Subjects under prospective evaluation showed significant positive associations between Δ C3 and Δ HOMA-IR. In earlier studies on protein-energy malnutrition, such as kwashiorkor and marasmus, serum concentrations of C3 were significantly lower than normal controls and were positively correlated with serum concentration of albumin and choline esterase (1,2). After diet therapy, the concentrations of C3 returned to the normal level (3). In our study, follow-up data were collected under unrestricted and free nutrition. Although BW and WC showed no significant change, fluctuations in insulin resistance may alter the concentration of C3.

Mets is characterized by chronic low-grade inflammation associated with moderately high concentrations of acute phase inflammatory proteins, such as TNF- α , IL-1, IL-6, and C1q (22). Weak but prolonged inflammation has been thought to accelerate coronary artery damage. Muscari et al. first identified a high concentration of serum C3 among patients with coronary vascular events (21). Their second report

indicated that the serum concentration of CRP, C3, and fibrinogen accurately predicts cardiovascular events in adults (23). In 2005, another cross-sectional study confirmed that complement C3 should be recognized as a coronary risk factor (24). In the normal bloodstream, the alternative complement pathway is capable of auto-activation because of a process termed “tickover” of C3 (spontaneously, at about 1% of total C3 per hour) (25). To clarify the importance of elevated C3 level, studies on larger numbers of cardiovascular patients are needed.

In conclusion, we have demonstrated that the serum concentration of C3 appears to be closely related to insulin resistance, especially in the metabolic syndrome, in Japanese patients.

REFERENCES

1. Hafez M, Aref GH, Mehareb SW, et al. Antibody production and complement system in protein energy malnutrition. *J Trop Med Hyg* 1977;80:36–39.
2. Haller L, Zubler RH, Lambert PH. Plasma levels of complement components and complement haemolytic activity in protein-energy malnutrition. *Clin Exp Immunol* 1978;34:248–252.
3. Keusch GT, Torun B, Johnson RB, Urrutia JJ. Impairment of haemolytic complement activation by both classical and alternative pathways in serum from patients with kwashiorkor. *Clin Lab Observ* 1984;105:434–436.
4. Colten HR, Ooi YM, Edelson PJ. Synthesis and secretion of complement proteins by macrophages. *Ann N Y Acad Sci* 1979;332:482–490.
5. Botto M, Lissandrini D, Sorio C, Walport MJ. Biosynthesis and secretion of complement component (C3) by activated human polymorphonuclear leukocytes. *J Immunol* 1992;149:1348–1355.
6. Brooimans RA, Stegmann AP, van Dorp WT, et al. Interleukin 2 mediates stimulation of complement C3 biosynthesis in human proximal tubular epithelial cells. *J Clin Invest* 1991;88:379–384.
7. Ohsawa I, Ohi H, Endo M, Fujita T, Kanmatsuse K, Nonaka M. Novel estimation of histologic activity in human glomerulonephritis by detection of complement component C3 messenger RNA. *Clin Exp Nephrol* 1998;2:50–57.
8. Naughton MA, Botto M, Carter MJ, Alexander GJ, Goldman JM, Walport MJ. Extrahepatic secreted complement C3 contributes to circulating C3 levels in humans. *J Immunol* 1996;156:3051–3056.
9. Choy LN, Rosen BS, Spiegelman BM. Adipsin and endogenous pathway of complement from adipose cells. *J Biol Chem* 1992;267:12736–12742.
10. Pomeroy C, Mitchel J, Eckert E, Raymond N, Crosby R, Dalmaso AP. Effect of body weight and caloric restriction on serum complement proteins, including factor D/adipsin: Studies in anorexia nervosa and obesity. *Clin Exp Immunol* 1997;108:507–515.
11. Weyer C, Tataranni PA, Pratley RE. Insulin action and insulinemia are closely related to the fasting complement C3, but not acylation stimulating protein concentration. *Diabetes Care* 2000;23:779–785.
12. Halkes CJM, van Dijk H, de Jaegere PPT, et al. Postprandial increase of complement component 3 in normolipidemic patients with coronary artery disease. Effects of expanded dose of simvastatin. *Arterioscler Thromb Vasc Biol* 2001;21:1526–1530.

13. Yu H, Yang Y, Zhang M, et al. Thyroid status influence on adiponectin, acylation stimulating protein (ASP) and complement C3 in hyperthyroid and hypothyroid subjects. *Nutr Metab (Lond)* 2006;10:3–13.
14. Razzouk L, Muntner P. Ethnic, gender, and age-related differences in patients with the metabolic syndrome. *Curr Hypertens Rep* 2009;11:127–132.
15. Ogden CL, Yanovski SZ, Carroll MD, Flegal KM. The epidemiology of obesity. *Gastroenterology* 2007;132:2087–2102.
16. Japanese Committee to Evaluate Diagnostic Standards for Metabolic Syndrome. Definition and the diagnostic standard for metabolic syndrome. *Nippon Naika Gakkai Zasshi* 2005;94:794–809 (in Japanese).
17. Matsuzawa Y. Metabolic syndrome-definition and diagnostic criteria in Japan. *J Atheroscler Thromb* 2005;12:301.
18. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Insulin resistance was assessed using the Homeostasis Model Assessment (HOMA): Fasting serum insulin ($\mu\text{U/mL}$) $\times$ fasting plasma glucose (mg/dL). Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentration in man. *Diabetologia* 1985;28:412–419.
19. Matsuo S, Imai E, Horio M, et al. Revised equations for estimated GFR from serum creatinine in Japan. *Am J Kidney Dis* 2009;53:982–992.
20. Van Harmelen V, Reynisdottir S, Cianflone K, et al. Mechanisms involved in the regulation of free fatty acid release from isolated human fat cells by acylation-stimulating protein and insulin. *J Biol Chem* 1999;274:18243–18251.
21. Yasruel Z, Cianflone K, Sniderman AD, Rosenbloom M, Walsh M, Rodriguez MA. Effect of acylation stimulating protein on the triacylglycerol synthetic pathway of human adipose tissue. *Lipids* 1991;26:495–499.
22. Tilg H, Moschen AR. Inflammatory mechanisms in the regulation of insulin resistance. *Mol Med* 2008;14:222–231.
23. Muscari A, Bastagli L, Poggiopollini G, et al. Different associations of C-reactive protein, fibrinogen, and C3 with traditional risk factors in middle-aged men. *Int J Cardiol* 2002;83:63–71.
24. Onat A, Uzunlar B, Hergenç G, et al. Cross-sectional study of complement C3 as a coronary risk factor among men and women. *Clin Sci* 2005;108:129–135.
25. Muller-Eberhard HJ. Molecular organization and function of the complement system. *Annu Rev Biochem* 1988;57:321–347.