Insulin Action and Insulinemia Are Closely Related to the Fasting Complement C3, but Not Acylation Stimulating Protein Concentration

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OBJECTIVE — An elevated C3 concentration has been reported in people with obesity, type 2 diabetes, hypertension, and dyslipidemia, and has been proposed to play a role in the development of atherosclerosis. We hypothesized that an elevated C3 concentration might be linked to insulin resistance and/or hyperinsulinemia, abnormalities commonly observed in association with the above conditions.

RESEARCH DESIGN AND METHODS — Fasting concentrations of C3 and acylation stimulating protein (ASP, C3adesarg), a cleavage product of C3 recently found to stimulate glucose uptake in vitro, were measured in 33 healthy nondiabetic Pima Indians (14 women and 19 men; age 27 ± 1 and body fat 33 ± 1%, means ± SEM). Subjects were characterized for body composition dual-energy X-ray absorptiometry, insulin action (insulin-stimulated glucose disposal [*M*], hyperinsulinemic glucose clamp), and glucose tolerance (75-g oral glucose tolerance test).

RESULTS — Fasting C3 and ASP concentrations were positively correlated ($r = 0.43$, $P < 0.05$). Fasting C3 concentration was closely related to percent body fat ($r = 0.77$), *M* ($r = -0.75$), and fasting insulin concentration ($r = 0.72$) (all $P < 0.0001$). Fasting C3 concentrations remained significantly related to *M* and fasting insulin after adjusting for percent body fat (partial $r = -0.53$ and 0.33, both $P < 0.05$). In subjects with impaired glucose tolerance, fasting C3 concentrations were higher than in those with normal glucose tolerance—a difference that remained after adjustment for percent body fat and *M*. We found that fasting ASP concentrations were significantly related to percent body fat ($r = 0.37$, $P < 0.05$), but not to *M* or fasting insulin.

CONCLUSIONS — In Pima Indians, fasting C3 concentration is closely related to adiposity, insulin action, and fasting insulin levels and may thus be a mediator for the postulated link between obesity, insulin resistance, hyperinsulinemia, and possibly atherosclerosis.

Diabetes Care 23:779–785, 2000

Adipose tissue is a metabolically active tissue that secretes a variety of paracrine and autocrine factors such as leptin, tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6). All of these factors are thought to be involved in the regulation of body weight and/or glucose homeostasis (1). Human adipocytes also synthesize and secrete the 3 proteins of the alternative complement pathway: complement C3, factor B, and factor D (adipsin) (2–11). The interaction of these 3 factors results in the generation of C3a, which, in vivo, is rapidly converted to C3adesarg, also termed acylation stimulating protein (ASP) (2–11).

Several lines of evidence suggest that both C3 and its cleavage product ASP may be linked to insulin resistance and/or hyperinsulinemia. Elevated C3 concentration levels have been reported in individuals with obesity (12), type 2 diabetes (13,14), hypertension (13), dyslipidemia (15), and coronary artery disease (14,16), all of which are known to be associated with insulin resistance. An elevated C3 concentration has also been found to predict myocardial infarction (17). This finding, along with the detection of C3 in atherosclerotic plaque (18), has led to the hypothesis that C3 may be an immune mediator in the development of atherosclerosis (18), which is typically accelerated in the above conditions. The notion that insulin resistance and/or hyperinsulinemia might be associated with an elevated C3 concentration is interesting for several reasons. First, it has been proposed recently that insulin resistance and type 2 diabetes may be, at least in part, a manifestation of a chronic activation of the innate immune system (19–21). Second, insulin resistance and hyperinsulinemia have long been proposed to be atherogenic risk factors (22,23). To date, the relationship between fasting C3 concentration and insulin action has not been established.

Another potential link between the alternative complement pathway and the insulin resistance syndrome has recently emerged from the observation that ASP stimulates glucose uptake in various cell types, including adipocytes (3), fibroblasts (4), and myotubes (5). Experimental data indicate that the effect of ASP on glucose transport is mediated, at least in part, through increased translocation of glucose transporters (GLUT1, GLUT3, and GLUT4) to the cell surface (4,5). In muscle (L6 myotubes), ASP appears to enhance the effect of insulin on glucose transport (5),

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Received for publication 17 December 1999 and accepted in revised form 17 February 2000.

Abbreviations: ASP, acylation stimulating protein (C3adesarg); EMBS, estimated metabolic body size; IGT, impaired glucose tolerance; IL-6, interleukin-6; *M*, insulin-stimulated glucose disposal; NGT, normal glucose tolerance; NIH, National Institutes of Health; TNF-α, tumor necrosis factor-α; WHO, World Health Organization.

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

Table 1—*Physical and metabolic characteristics of the study population*

Characteristic	All	By sex		By glucose tolerance	
		Women	Men	NGT	IGT
n	33	14	19	25	8
Age (years)	27 ± 1	27 ± 1	27 ± 2	27 ± 1	27 ± 2
Body weight (kg)	97.6 ± 3.9	94.0 ± 6.4	100.2 ± 5.0	96.7 ± 4.5	100.2 ± 8.4
BMI (kg/m^2)	34.2 ± 1.3	36.0 ± 2.3	32.9 ± 1.4	33.5 ± 1.4	36.7 ± 2.9
Body fat (%)	33 ± 1	38 ± 1.2	29 ± 1*	32 ± 1	36 ± 3
Fat mass (kg)	32.4 ± 2.1	37.0 ± 3.3	29.0 ± 2.5†	31.4 ± 2.3	35.2 ± 4.7
Fat-free mass (kg)	65.2 ± 2.4	57.1 ± 3.2	71.2 ± 2.8‡	65.3 ± 2.7	65.0 ± 5.3
Waist-to-thigh ratio	1.71 ± 0.05	1.64 ± 0.07	1.75 ± 0.06	1.67 ± 0.05	1.82 ± 0.12
Fasting glucose (mmol/l)	4.9 ± 0.5	4.9 ± 0.8	5.0 ± 0.7	4.9 ± 0.5	5.0 ± 0.8
Fasting insulin (pmol/l)	252 ± 12	294 ± 24	216 ± 12‡	240 ± 18	282 ± 21
2-h glucose (mmol/l)	6.4 ± 0.3	6.9 ± 0.5	6.1 ± 0.4	5.6 ± 0.2	9.0 ± 0.3*
2-h insulin (pmol/l)	936 ± 96	1,212 ± 156	732 ± 102†	792 ± 96	1,386 ± 204†
M (mg · kg^{-1}EMBS · min^{-1})	2.6 ± 0.1	2.4 ± 0.1	2.8 ± 0.2	2.8 ± 0.2	2.2 ± 0.1†
Fasting plasma C3 (mg/dl)	171 ± 4	180 ± 6	165 ± 6	165 ± 5	189 ± 6‡
Fasting plasma ASP (ng/ml)	277 ± 16	304 ± 24	261 ± 22	265 ± 17	323 ± 36

Data are *n* or means ± SEM. Symbols indicate significant differences between women and men or between individuals with NGT and IGT (*$P < 0.001$, †$P < 0.05$, ‡$P < 0.01$, unadjusted comparison).

which suggests that ASP may play an independent role in the regulation of insulin-stimulated glucose uptake. These in vitro findings have been supported most recently by the demonstration that the intraperitoneal injection of human ASP into C57BL/6 mice reduced glucose excursions in response to an oral fat load (6). As with C3, elevated fasting ASP concentrations have been reported in individuals with obesity (7,8) and coronary artery disease (9); no data are available, however, on the relationship between fasting ASP concentration and insulin action in humans.

The aim of the present study was to examine the relationship of fasting C3 and ASP concentrations to adiposity, insulin action, and insulinemia in nondiabetic Pima Indians.

RESEARCH DESIGN AND METHODS

Study subjects

We included 33 Pima Indians in this analysis (Table 1). All subjects were participants in an ongoing longitudinal study of the pathogenesis of type 2 diabetes (24). At the time of the study, all were between 20 and 50 years of age; nondiabetic according to a 75-g oral glucose tolerance test (using World Health Organization [WHO] and American Diabetes Association criteria); nonsmokers; and considered healthy after a physical examination and routine laboratory tests. No subject had clinical or laboratory signs of acute infection. None had a personal history of hypertension, dyslipidemia, atherosclerotic disease, autoimmune disease, or other condition known to be associated with altered C3 or ASP concentrations. Participants were admitted for 8–10 days to the National Institutes of Health (NIH) Clinical Research Unit in Phoenix, Arizona, where they were fed a weight-maintaining diet (50% of calories as carbohydrate, 30% as fat, and 20% as protein) and instructed to abstain from strenuous exercise. After being on the diet for at least 3 days, participants underwent a series of tests for the assessment of body composition, glucose tolerance, and insulin action. The protocol was approved by the Tribal Council of the Gila River Indian Community and by the Institutional Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases. All subjects provided written informed consent before participating in the study.

Anthropometric measurements

Body composition was estimated by total-body dual-energy X-ray absorptiometry (DPX-L; Lunar Radiation, Madison, WI) with calculation of percent body fat, fat mass, and fat-free mass as previously described (25). Waist and thigh circumferences were measured at the umbilicus and the gluteal fold in the supine and standing positions, respectively, and the waist-to-thigh ratio was calculated as an index of body fat distribution.

Oral glucose tolerance test

After a 12-h overnight fast, subjects underwent a 75-g oral glucose tolerance test. Baseline blood samples were drawn for determination of serum C3 concentration, and plasma ASP, glucose, and insulin concentrations using prechilled syringes and prechilled glass tubes (EDTA [no addition of Futhan] for glucose, insulin, and ASP). Plasma glucose concentrations were also measured 2 h after glucose ingestion to assess glucose tolerance according to the 1985 WHO diagnostic criteria.

Hyperinsulinemic-euglycemic glucose clamp

Insulin action was assessed by a hyperinsulinemic-euglycemic glucose clamp as described (24). In brief, after an overnight fast, a primed continuous intravenous insulin infusion was administered for 100 min at a constant rate of 40 mU · m^{-2} · min^{-1}, which achieved a steady-state plasma insulin concentration of 840 ± 23 pmol/l (means ± SEM). Plasma glucose concentrations were maintained at ~5.5 mmol/l with a variable infusion of a 20% glucose solution. Using both the rate of glucose infused during the last 40 min of the clamp and the rate of endogenous glucose output (measured by a primed [30 μCi], continuous [0.3 μCi per min] [3-^{3}H]glucose infusion), the rate of total insulin-stimulated glucose disposal (*M*) was calculated and normalized to estimated metabolic body size (EMBS, fat-free mass

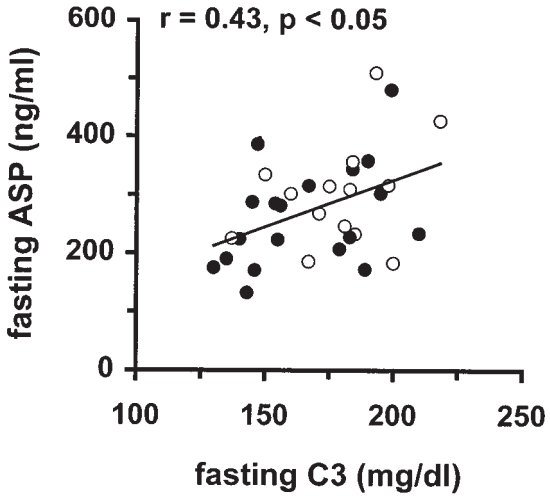

Figure 1—*Relationship between fasting ASP and C3 concentrations in 33 nondiabetic Pima Indians.* ●*, Men;* ○*, women.*

+ 17.7 kg) (24). To determine the acute effect of hyperinsulinemia on C3 levels, plasma C3 concentrations were measured at baseline and after the clamp in a separate group of 10 nondiabetic Pima Indians.

Analytic procedures

Plasma glucose concentrations were determined by the glucose oxidase method (Beckman Instruments, Fullerton, CA), and plasma insulin concentrations were determined by an automated immunoassay (Access; Beckman Instruments). Serum C3 concentrations were measured by an immunoturbidimetric assay (Cobas Integra; Roche Diagnostics, Indianapolis, IN), which used a rabbit anti-C3 antibody specific for human C3 (normal range 90–180 mg/dl; coefficient of variation 2%). Serum was separated promptly from the clot and analyzed within 48 h of sampling. Plasma ASP concentrations were measured by a radioimmunoassay (Biotrak; Amersham, Buckinghamshire, U.K.), which used a rabbit anti-C3adesarg (ASP) antibody specific for human ASP and avoided interference with the precursor (C3) by a selective precipitation step (normal range 70–500 ng/ml, coefficient of variation 3–9%). Plasma was stored at −20°C and analyzed within 4 weeks of sampling.

Statistical analyses

Statistical analyses were performed using the software of the SAS Institute (Cary, NC). Log-transformed values of *M*, fasting insulin concentration, and 2-h insulin concentration were used for all statistical analyses to achieve normal distributions. The relationship of fasting C3 and ASP concentrations to anthropometric and metabolic variables were assessed by simple linear regression models. Partial correlation analyses were used to examine the relationship between variables after adjustment for covariates. Multiple linear regression models were used to compare anthropometric and metabolic variables between females and males and between individuals with normal glucose tolerance (NGT) and impaired glucose tolerance (IGT), with and without (Table 1) adjustment for covariates. Stepwise multiple regression analyses were used both to identify independent determinants of fasting C3 and *M* and to calculate the percentage of variance (R^2) explained by those. A paired *t* test was used to determine whether the serum C3 concentration changed during the insulin infusion.

RESULTS

Complement C3

Fasting ASP and C3 concentrations were positively correlated (Fig. 1). The fasting C3 concentration was positively correlated with percent body fat, fasting insulin concentration, and 2-h glucose concentration, and negatively correlated with *M* (Fig. 2). Significant positive correlations were also found between fasting C3 and the following: BMI ($r = 0.78$, $P < 0.001$), waist-to-thigh ratio ($r = 0.43$, $P < 0.05$), fasting glucose concentration ($r = 0.47$, $P < 0.01$), and 2-h insulin concentration ($r = 0.58$, $P < 0.001$). The relationship between fasting C3 and waist-to-thigh ratio was no longer significant after adjustment for percent body fat (partial $r = 0.17$, NS). In contrast, fasting C3 remained related to *M* and fasting insulin after adjusting for percent body fat (partial $r = -0.53$ and 0.33, respectively, both $P < 0.05$). In a multiple regression analysis with *M* as the dependent variable, fasting insulin and fasting C3 were independently related to *M*, explaining 60 and 7% of the variance (R^2) in *M*, respectively. Percent body fat was not a significant determinant of *M* when C3 was included in the model. Between males and females, unadjusted fasting C3 did not differ significantly (Table 1); after adjusting for age, percent body fat, and *M*, however, fasting C3 was higher in males than in females (+17 ± 6 mg/dl, $P < 0.01$). Fasting C3 was higher in individuals with IGT than in those with NGT (Table 1), a difference that became smaller, but remained significant, after adjusting for age, sex, percent body fat, and *M* (+11 ± 5 mg/dl, $P < 0.05$). In a multiple regression analysis with C3 as the dependent variable, percent body fat, *M*, sex, and glucose tolerance status were all independent determinants, explaining a total of 80% of the variance in fasting C3. Mean serum C3 concentration was unchanged after 100 min of euglycemic hyperinsulinemia (Fig. 3).

Acylation stimulating protein

The fasting ASP concentration was positively correlated with percent body fat, but not with *M*, fasting insulin concentration, or 2-h glucose concentration (Fig. 2). No significant correlations were found between fasting ASP and BMI ($r = 0.26$, NS), waist-to-thigh ratio ($r = 0.05$, NS), fasting glucose concentration ($r = 0.02$, NS), or 2-h insulin concentration ($r = 0.27$, NS). Mean fasting ASP did not differ between males and females or between individuals with IGT and NGT (Table 1), regardless of whether values were adjusted for age, sex, and percent body fat.

CONCLUSIONS

— Several cytokines secreted by adipose tissue, such as TNF-α and IL-6, have been proposed to mediate, at least in part, the relationship between obesity and insulin resistance (1,19,26–29). Growing evidence suggests that complement C3, another adipocyte-derived cytokine, and its cleavage product, ASP (C3adesarg), might also be linked to insulin resistance (2–17). In the present study, we examined the relationship of fasting C3 and

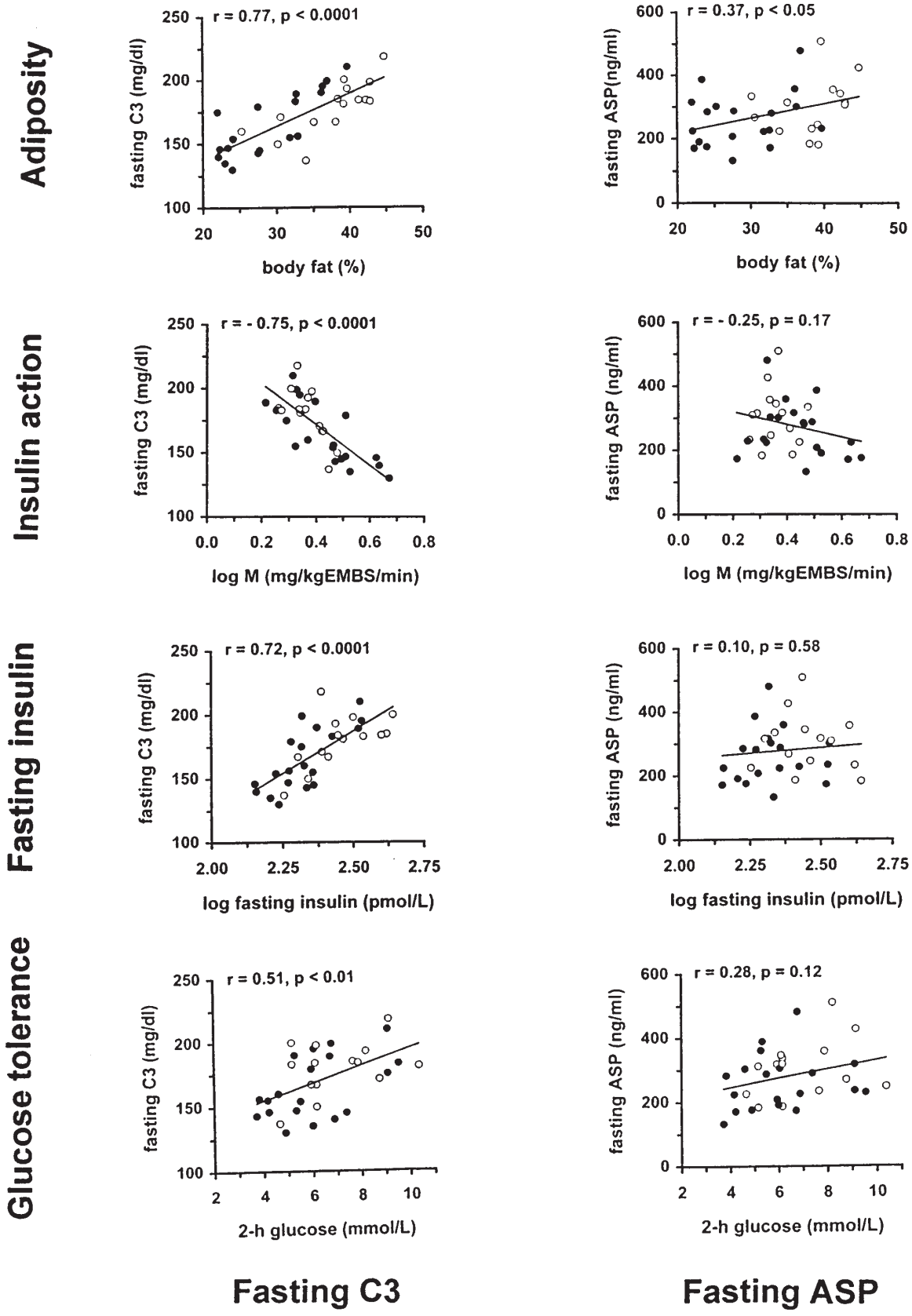

Figure 2—*Relationship of the fasting C3 (left) and ASP (right) concentrations to percent body fat (first row), insulin-stimulated glucose disposal (second row), fasting insulin concentration (third row), and 2-h glucose concentration (fourth row) in 33 nondiabetic Pima Indians. ●, Men; ○, women.*

ASP concentrations to adiposity, insulinemia, and insulin action as directly measured by the euglycemic-hyperinsulinemic clamp technique. We found that fasting C3 concentration was correlated not only with adiposity, but also closely and independently with insulin action and fasting insulin concentration. Although fasting C3 and fasting ASP concentrations were positively related to one another, fasting ASP concentration was related only to adiposity, and not to insulin action or insulinemia.

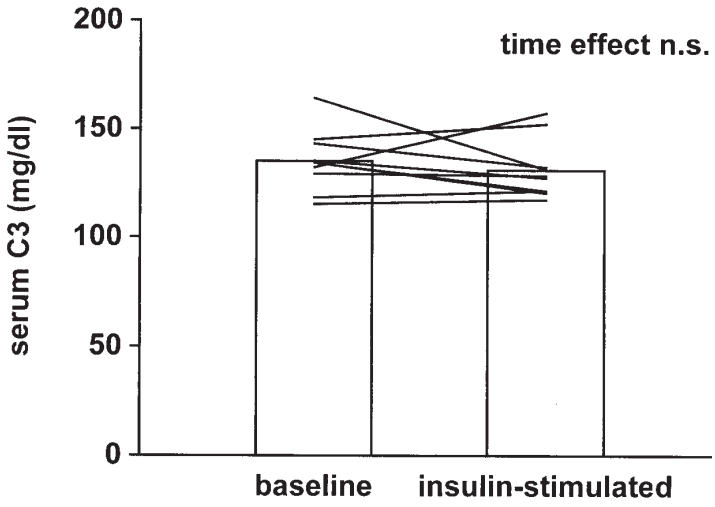

Figure 3—*Mean (bars) and individual (lines) C3 concentration before and at the end of 100 min of euglycemic hyperinsulinemia (plasma insulin concentration 840 ± 252 pmol/l [means ± SD]) in 10 nondiabetic Pima Indians.*

Complement C3

Individuals with obesity are known to have an elevated fasting C3 concentration (12). Our finding of a positive relationship between fasting C3 concentration and percent body fat was therefore not surprising. It remains unknown whether this association is simply attributable to an increased secretion of C3 from enlarged body fat stores or due to secondary mechanisms involving hepatic C3 production. Elevated C3 concentrations also have been reported in individuals with type 2 diabetes (13,14), hypertension (13), and dyslipidemia (15), even in the absence of obesity. The present finding that fasting C3 concentration is closely correlated with insulin action and fasting insulin concentration independent of adiposity suggests that insulin resistance and/or hyperinsulinemia may be common mechanisms underlying elevated C3 concentrations in these conditions. The fasting C3 concentration was also positively related to 2-h glucose concentration and, accordingly, was elevated in individuals with impaired, but not yet diabetic, glucose tolerance.

The present association study does not resolve whether an elevated C3 concentration is a cause or a consequence of either insulin resistance and/or hyperinsulinemia. On one hand, C3 could be a pathogenic factor in the development of insulin resistance by either direct or indirect effects on the insulin signaling cascade, such as demonstrated for TNF-α (26,27). On the other hand, an elevated C3 concentration may be secondary to insulin resistance and/or hyperinsulinemia. It is known, for instance, that C3 mRNA expression is upregulated by TNF-α and IL-6 (30), other adipocyte-derived cytokines implicated in the pathophysiology of insulin resistance (26–29). Glucocorticoids, which in excess lead to abdominal obesity, insulin resistance, and glucose intolerance, also increase C3 expression in vitro (31). Finally, insulin itself has been shown to have a regulatory effect on C3 expression (32,33) and thereby may directly contribute to elevated C3 concentrations in individuals with obesity and insulin resistance. Interestingly, although insulin increases C3 expression in adipose tissue (32), it was found to inhibit C3 expression in rat hepatoma cells (33). It is not known whether the lack of effect of short-term euglycemic hyperinsulinemia on the prevailing C3 concentration in the present study is attributable to this divergent effect of insulin on C3 expression, or simply due to insufficient study duration.

Regardless of the underlying mechanism, our finding that the fasting C3 concentration is closely related to insulin action and insulinemia may have pathophysiological and clinical relevance.

First, it recently has been suggested that insulin resistance, and ultimately type 2 diabetes, may in part be a manifestation of a chronic acute-phase response, i.e., a reaction of the innate immune system's response to stressful stimuli that could include overnutrition, physical inactivity, and aging (19–21). This concept was largely developed from the observation that plasma concentrations of other inflammatory cytokines and acute-phase proteins such as TNF-α (28), IL-6 (29), and/or C-reactive protein (19–21) are increased in individuals with obesity and type 2 diabetes. To our knowledge, the present study is the first to demonstrate a close relationship between one of these inflammatory cytokines, C3, and insulin action as measured directly by the hyperinsulinemic clamp technique.

Second, it has been suggested that an elevated C3 concentration may play a causative role in the development of atherosclerosis (18,34). This hypothesis is supported by the findings that an elevated C3 concentration predicts myocardial infarction (17) and that C3 accumulates in atherosclerotic plaques (18). Our finding of a close relationship between C3 and insulin action suggests that C3 might be a mediator for the postulated link between insulin resistance and/or hyperinsulinemia and atherosclerosis (22,23). It is important to note, however, that although Pima Indians are characterized by a very high prevalence of obesity, insulin resistance, hyperinsulinemia, and type 2 diabetes, their propensity to atherosclerotic disease is much lower than expected (8). Studies in other populations are therefore warranted to test whether our findings in Pima Indians can be confirmed in other ethnic groups and to further elucidate the potential role of C3 as a mediator between insulin resistance, hyperinsulinemia, and atherosclerosis. Preliminary findings by Muscari et al. (35), who studied a large group of Caucasian subjects, indicate that the fasting insulin concentration is correlated with C3 in other populations as well.

Acylation stimulating protein

As with C3, elevated ASP concentrations have been reported in individuals with obesity (7,8) and coronary artery disease (9). The results of the present study indicate that

fasting ASP concentration is positively related to adiposity and to fasting C3 concentration, but not to insulin action or insulinemia. The relationship between fasting ASP and C3 concentrations was rather weak, but stronger than the relationship between fasting ASP and adiposity. This finding suggests that the correlation between adiposity and ASP concentration (7,8) may be mediated by increased C3 secretion. In contrast, fasting adipsin and ASP concentrations appear to be unrelated (10). The finding that fasting ASP concentration was unrelated to insulin action does not exclude a possible role of ASP in the regulation of glucose uptake in humans. As previously pointed out by us (8) and others (2), the action of ASP is a function not only of the prevailing ASP concentration, but also of the sensitivity of target tissues to ASP. Fibroblasts from some patients with hyperapo(b)-lipoproteinemia, for instance, are resistant to the effect of ASP (11). Based on these and other findings, it has been proposed that ASP unresponsiveness might contribute to metabolic disturbances associated with abdominal obesity (2).

In summary, the present study revealed that in Pima Indians, insulin action and insulinemia are closely related to fasting complement C3, but not ASP, concentration. Although the exact biological mechanisms underlying these associations remain to be elucidated, our data suggest that complement C3 may be a mediator for the postulated link between obesity, insulin resistance, hyperinsulinemia, and possibly atherosclerosis.

Acknowledgments — We gratefully acknowledge the help of the nursing staff and of Dr. Arline Salbe and the dietary staff for the care of the volunteers. We also thank the technical staff, particularly Ms. Linda Phillips, for assisting in the laboratory analyses, and Beth Hauth and Dr. Robert Evans from the University of Pittsburgh for the measurement of ASP. Finally, we are grateful to the members and leaders of the Gila River Indian Community for their continuing cooperation in our studies.

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