Inflammation, Insulin Resistance, and Adiposity

A study of first-degree relatives of type 2 diabetic subjects

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OBJECTIVE — Inflammatory markers such as C-reactive protein (CRP) are associated with insulin resistance, adiposity, and type 2 diabetes. Whether inflammation causes insulin resistance or is an epiphenomenon of obesity remains unresolved. We aimed to determine whether first-degree relatives of type 2 diabetic subjects differ in insulin sensitivity from control subjects without a family history of diabetes, whether first-degree relatives of type 2 diabetic subjects and control subjects differ in CRP, adiponectin, and complement levels, and whether CRP is related to insulin sensitivity independently of adiposity.

RESEARCH DESIGN AND METHODS — We studied 19 young normoglycemic nonobese first-degree relatives of type 2 diabetic subjects and 22 control subjects who were similar for age, sex, and BMI. Insulin sensitivity (glucose infusion rate [GIR]) was measured by the euglycemic-hyperinsulinemic clamp. Dual-energy X-ray absorptiometry determined total and abdominal adiposity. Magnetic resonance imaging measured abdominal adipose tissue volumes.

RESULTS — First-degree relatives of type 2 diabetic subjects had a 20% lower GIR than the control group (51.8 ± 3.9 vs. 64.9 ± 4.6 $\mu mol \cdot min^{-1} \cdot kg$ fat-free $mass^{-1}$, $P = 0.04$). However, first-degree relatives of subjects with type 2 diabetes and those without a family history of diabetes had normal and comparable levels of CRP, adiponectin, and complement proteins. When the cohort was examined as a whole, CRP was inversely related to GIR ($r = -0.33$, $P = 0.04$) and adiponectin ($r = -0.34$, $P = 0.03$) and positively related to adiposity ($P < 0.04$). However, CRP was not related to GIR independently of fat mass. In contrast to C3 ($r = 0.41$, $P = 0.009$) and factor B ($r = 0.43$, $P = 0.005$), CRP was unrelated to factor D.

CONCLUSIONS — The insulin-resistant state is not associated with changes in inflammatory markers or complement proteins in subjects at high risk of type 2 diabetes. Our study confirms a strong relationship between CRP and fat mass. Increasing adiposity and insulin resistance may interact to raise CRP levels.

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Insulin resistance is fundamental to the pathogenesis of the metabolic syndrome and type 2 diabetes (1) and is a heritable trait (2). Type 2 diabetes is also strongly genetically determined (3). Consequently, first-degree relatives of subjects with type 2 diabetes demonstrate the metabolic accompaniments of insulin resistance before they develop overt diabetes (4). Because hyperglycemia further impairs both insulin action and insulin secretion (5), the study of primary metabolic defects leading to insulin resistance is best undertaken before insulin secretion begins to fail and blood glucose rises (6). In addition to genetic factors, excess body fat, particularly visceral fat, has been linked to the pathogenesis of insulin resistance and type 2 diabetes. We have reported that increased central adiposity is strongly related to reduced insulin sensitivity in subjects with and without first-degree relatives with type 2 diabetes (7). Furthermore, we, along with other researchers, have hypothesized that excess lipid supply, via skeletal muscle triglyceride (or other lipid moieties such as diacylglycerols), is responsible for insulin resistance and may contribute to insulin secretory failure in type 2 diabetes (8–10).

Recently, "inflammation" and "inflammatory" cytokines have been postulated to be important additional pathogenetic factors in the development of insulin resistance and type 2 diabetes (11,12). C-reactive protein (CRP), a nonspecific marker of the inflammatory response (13), is most consistently associated with the development of type 2 diabetes (14–16); however, a causal association remains unproven. CRP is known to activate the classical complement pathway (17), and this has been proposed as a mechanism of tissue damage in myocardial infarction (18). Whether elevations of CRP, albeit within the normal range and/or "inflammation" cause insulin resistance, or rather, are somehow an epiphenomenon of obesity itself, remains unresolved.

Adiponectin, a recently described protein secreted from adipocytes, has been reported to have anti-inflammatory, antidiabetic, and antiatherogenic properties that are probably partly mediated through downregulation of circulating CRP and the inflammatory response (19). Several reports, including the recent iden-

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Abbreviations: CRP, C-reactive protein; DXA, dual-energy X-ray absorptiometry; FFM, fat-free mass; GIR, glucose infusion rate; IL-6, interleukin-6; MRI, magnetic resonance imaging.

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

tification of adiponectin receptors in skeletal muscle and liver (20), have indicated that adiponectin may play an integral role in the pathogenesis of insulin resistance associated with obesity. In support of this suggestion, insulin sensitivity is directly, and adiposity inversely, associated with circulating adiponectin levels (21), and hypoadiponectinemia has been reported to predict the development of type 2 diabetes in healthy subjects (22).

To examine the primary defects associated with insulin resistance in the absence of glucotoxicity, we compared young, healthy, nonobese subjects with a strong family history of type 2 diabetes (REL) with control subjects without any family history of diabetes (CON) who were similar for age, sex, and BMI. These subjects were specifically studied because they had normal fasting lipid levels and normal glucose tolerance. We aimed to determine whether nonobese normoglycemic REL subjects differed in insulin sensitivity from CON subjects. If so, we aimed to investigate if the REL and CON groups differed in CRP and adiponectin levels and whether REL subjects showed features of pathological complement activation (as indicated by decreased plasma levels) or evidence of acute-phase stimulation (as shown by an increase in acute-phase components, C4, C3, and factor B). In the overall group, we also investigated whether CRP concentrations were related to insulin sensitivity independent of adiposity (total or abdominal).

RESEARCH DESIGN AND METHODS

A total of 41 healthy, nonobese, sedentary, nonsmoking volunteers (22 CON and 19 REL subjects, aged 20–45 years, without history of cardiovascular disease, hypertension, endocrine disorders, or dyslipidemia) were recruited from advertisements in newspapers and on campus. The REL group (14 women and 5 men) had two first-degree relatives (at least one parent) with type 2 diabetes or one first-degree relative and a personal history of gestational diabetes. The CON group (12 women and 10 men) had no known family history of diabetes. Each subject's weight was stable (<2-kg change) for the 3 months before study entry. All women were premenopausal and not pregnant at the time of the study. All subjects had normal glucose tolerance by oral glucose tolerance testing. There was no significant difference in routine physical activity (exercise/home duties) undertaken by the subjects (based on a frequency questionnaire). The St. Vincent's Hospital human research ethics committee approved the study, and all subjects provided written informed consent.

Procedures were undertaken following a 10-h overnight fast. Subjects were asked to refrain from alcohol and strenuous exercise for 24 h before testing. Fasting blood was collected for plasma insulin, glucose, lipids, CRP, adiponectin, and complement proteins. A 120-min euglycemic-hyperinsulinemic clamp was performed to determine whole-body insulin sensitivity (glucose infusion rate [GIR]). Body composition was assessed by dual-energy X-ray absorptiometry (DXA) (Lunar DPX; GE-Lunar, Madison, WI; software version 1.35y) and abdominal fat volume by magnetic resonance imaging (MRI).

Body composition

Weight was recorded to the nearest 0.1 kg using a digital scale with the subject wearing only a hospital gown and height to the nearest 0.5 cm using a stadiometer. Waist circumference was determined at the level of the umbilicus and hip circumference at the level of the greater trochanters to the nearest 0.5 cm using a steel tape measure. BMI was calculated as weight divided by height squared (kg/m^2) and waist-to-hip ratio by dividing waist circumference by hip circumference. Whole-body DXA was used to analyze body composition according to a three-compartmental model comprising fat mass, lean tissue, and bone mineral content. Fat-free mass (FFM) is defined as the sum of lean tissue and bone mineral content. The method of central body fat measurement has been described and includes both visceral and some subcutaneous abdominal fat (7). Four T1-weighted axial abdominal MRI scans (5 mm thickness, echo time 14 ms, repetition time 500 ms) were performed between levels of L1/2 and L4/5 intervertebral discs. Imaging software (NIH Image 1.62; National Institutes of Health, Bethesda, MD) was used to quantitate areas and volumes of visceral abdominal adipose tissue and subcutaneous abdominal adipose tissue as previously described by our group (23). Scans were unavailable for one CON subject due to claustrophobia and four REL subjects due to technical difficulties.

Euglycemic-hyperinsulinemic clamp

Intravenous cannulae were placed in each forearm, one for glucose and insulin infusions and the other retrogradely in a warmed forearm for arterialized venous blood collection. Insulin was infused at 50 $mU \cdot m^{-2} \cdot min^{-1}$ (shown previously to suppress hepatic glucose output [24]) for 120 min, achieving serum insulin levels of 115 ± 4 mU/l. Plasma glucose was measured every 10 min (YSI 2300 StatPlus; YSI, Yellow Springs, OH), and the GIR was adjusted to maintain plasma glucose at 5 mmol/l. The GIR was calculated over the last 30 min of the clamp and adjusted for FFM (units: $\mu mol \cdot min^{-1} \cdot kg\ FFM^{-1}$).

Biochemical assays

CRP was measured by a highly sensitive assay, using a Dade Behring Dimension RxL Chemistry Analyzer with reagents and calibrators supplied by Dade Behring Diagnostics (Sydney, Australia). The CRP method is based on a particle-enhanced turbidimetric immunoassay technique. Latex particles coated with antibody to CRP aggregate in the presence of CRP in the sample. The increase in the turbidity that accompanies aggregation is proportional to the CRP concentration. The inter- and intra-assay coefficients of variation (CVs) for this assay were <6%, and the analytical range was 0.5–500 mg/l. C3 and C4 were quantitated by standard nephelometry (Beckman, Sydney, Australia) and C1q and factor B by radial immunodiffusion using monospecific polyclonal rabbit antibodies (Dako, Sydney, Australia) and a binding site standard (U.K.). Factor D was measured by in-house sandwich enzyme-linked immunosorbent assay, using pure factor D (Calbiochem, Melbourne, Australia) as a standard and antibodies from the binding site. Adiponectin levels were assayed by commercial radioimmunoassay (Linco Research, St. Charles, MO). Fasting serum was analyzed for insulin by radioimmunoassay (Linco Research); total cholesterol, HDL cholesterol, and triglycerides by enzymatic colorimetry (Roche, Indianapolis, IN); and nonesterified fatty acids by enzymatic colorimetry (Wako, Osaka, Japan). Inter- and intra-assay CVs were <10% for these assays in our laboratory.

Table 1—*Cohort characteristics and biochemical variables* (n = *41*)

Variables	REL group	CON group	*P*
n (women/men)	14/5	12/10	
Age (years)	30.7 ± 1.6	31.3 ± 1.6	0.80
Weight (kg)	73.4 ± 4.1	73.7 ± 3.5	0.90
BMI (kg/m^2)	25.8 ± 1.1	24.3 ± 0.8	0.29
Waist (cm)	80.0 ± 3.2	78.7 ± 2.6	0.76
Waist-to-hip ratio	0.79 ± 0.02	0.82 ± 0.02	0.38
Total body fat (kg) (DXA)	25.5 ± 2.5	20.9 ± 2.0	0.15
FFM (kg) (DXA)	47.7 ± 2.6	52.9 ± 2.9	0.20
Central abdominal fat (kg) (DXA)	1.39 ± 0.15	1.21 ± 0.13	0.37
Subcutaneous abdominal fat (l) (MRI)*	2.23 ± 0.29	1.69 ± 0.25	0.17
Visceral abdominal fat (l) (MRI)*	0.59 ± 0.07	0.61 ± 0.11	0.88
Fasting plasma glucose (mmol/l)	5.0 ± 0.1	4.9 ± 0.1	0.35
2-h postchallenge glucose (mmol/l)†	5.8 ± 0.2	5.2 ± 0.3	0.12
Fasting plasma insulin (mU/l)	10.6 ± 0.8	8.5 ± 0.9	0.08
GIR (μmol · min^{-1} · kg FFM^{-1})	51.8 ± 3.9	64.9 ± 4.6	0.04
Fasting total cholesterol (mmol/l)	4.08 ± 0.22	4.19 ± 0.18	0.71
Fasting HDL cholesterol (mmol/l)	0.89 ± 0.07	0.90 ± 0.06	0.93
Fasting triglycerides (mmol/l)	0.81 ± 0.11	0.68 ± 0.05	0.27
Fasting NEFAs (mmol/l)	0.46 ± 0.04	0.42 ± 0.05	0.58
CRP (mg/l)	1.55 ± 0.23	1.68 ± 0.46	0.33
Adiponectin (μg/ml)	14.6 ± 1.7	15.1 ± 1.4	0.82
C1q (mg/l)	103 ± 4	101 ± 2	0.67
C4 (mg/l)	218 ± 15	217 ± 16	0.96
C3 (g/l)	1.21 ± 0.07	1.05 ± 0.05	0.06
Factor B (mg/l)	263 ± 17	227 ± 13	0.10
Factor D (μg/ml)	1.39 ± 0.09	1.33 ± 0.06	0.58

Data are mean ± SE. *n = 36; †following 75-g oral glucose tolerance test. NEFA, nonesterified fatty acid.

Statistical analysis

Data are expressed as mean ± SE. Comparisons between groups were performed using one-way ANOVA for continuous variables and χ^2 tests for categorical variables. Relationships among continuous variables were assessed by simple and multiple regression. The appropriateness of the multiple regression models was examined using a runs test of the sign of the residuals ordered on predicted values. For all models, *P* values were >0.2, indicating that the data are scattered randomly about the model predictions. Due to its skewed distribution, CRP was $\log_{10}$ transformed before all analyses. Data were analyzed using Statview 5 (SAS Institute, Cary, NC). $P < 0.05$ was considered significant.

RESULTS

Comparison of adiposity, metabolic parameters, and adiponectin, complement and CRP levels in REL and CON groups

Cohort characteristics are shown in Table 1. Age and sex distribution were similar in the REL and CON groups ($P = 0.80$ and $P = 0.20$, respectively). Both groups had similar anthropometric and direct measures of adiposity and similar levels of fasting glucose, insulin, and lipids (Table 1). REL subjects were ~20% less insulin sensitive than CON subjects (GIR 51.8 ± 3.9 vs. 64.9 ± 4.6 μmol · min^{-1} · kg FFM^{-1}, respectively, $P = 0.04$). However, adiponectin and proteins of classical (C1q and C4) and alternative (C3, factors B and D) complement pathways were not significantly different between groups (Table 1). Levels of all complement proteins were in the normal range, with no evidence of classical or alternative pathway component consumption or acute-phase stimulation.

There was no significant difference in CRP levels between the REL and CON groups (1.55 ± 0.23 vs. 1.68 ± 0.46 mg/l, respectively, $P = 0.33$). Despite nonsignificantly more women in the REL group (which could have biased the REL group toward greater insulin sensitivity and higher CRP levels), this group was less insulin sensitive than the CON group and had comparable CRP levels. Differences between the groups were similar when only women were studied ($n = 26$), with no difference in complement proteins ($P > 0.18$) or CRP ($P = 0.58$), despite GIR being 23% lower in REL women (48.9 ± 5.0 vs. 63.5 ± 5.8 μmol · min^{-1} · kg FFM^{-1}, respectively, $P = 0.068$).

CRP in relation to adiposity; fasting lipid; glucose, insulin, and adiponectin levels; and insulin sensitivity

When the group was examined as a whole, inverse correlations were found between whole-body insulin sensitivity (GIR) and measures of adiposity: BMI ($r = -0.28$, $P = 0.08$), total body fat ($r = -0.41$, $P = 0.007$), and central abdominal fat ($r = -0.43$, $P = 0.006$). CRP was directly related to BMI, total body fat, and central abdominal fat by DXA and visceral abdominal adipose tissue and subcutaneous abdominal adipose tissue by MRI (Table 2).

CRP levels were significantly correlated with fasting triglycerides ($r = 0.39$, $P = 0.01$) but not with other lipid levels (total cholesterol, HDL cholesterol, or nonesterified fatty acids) (Table 2). While all subjects had normal glucose tolerance, CRP levels were positively associated with fasting plasma glucose ($r = 0.32$, $P = 0.045$) but not with fasting insulin (Table 2). CRP was inversely related to fasting adiponectin ($r = -0.34$, $P = 0.03$).

Circulating levels of CRP were inversely associated with GIR ($r = -0.33$, $P = 0.04$) (i.e., elevated CRP was associated with reduced insulin sensitivity). However, in a multiple regression model, with insulin sensitivity (GIR) as the dependent variable, CRP was not significantly related to GIR ($P = 0.19$) independently of total body fat ($P = 0.03$) ($r^2 = 0.21$, $P = 0.01$ for model). Similar results were found when measures of abdominal adiposity were substituted for total body fat, including central fat by DXA ($P = 0.04$; CRP: $P = 0.29$) and subcutaneous abdominal adipose tissue by MRI ($P = 0.009$; CRP: $P = 0.74$).

CRP in relation to complement protein levels

In the combined group, CRP was significantly related to C3 ($r = 0.41$, $P = 0.009$) and factor B ($r = 0.43$, $P = 0.005$), the acute-phase components of the alterna-

Table 2—*Simple linear regression analyses between CRP levels and adiposity measures, metabolic variables, adiponectin, and complement proteins* (n = 41)

Variables	*r*	*P*
Age (years)	0.12	0.45
Weight (kg)	0.34	0.03
BMI (kg/m^2)	0.41	0.008
Waist (cm)	0.36	0.02
Waist-to-hip ratio	0.12	0.46
Total body fat (kg) (DXA)	0.35	0.03
FFM (kg) (DXA)	0.15	0.35
Central abdominal fat (kg) (DXA)	0.43	0.005
Subcutaneous abdominal fat (L) (MRI)*	0.59	0.0002
Visceral abdominal fat (L) (MRI)*	0.38	0.02
Fasting plasma glucose (mmol/l)	0.32	0.045
2-h postchallenge glucose (mmol/l)†	0.03	0.88
Fasting plasma insulin (mU/l)	0.26	0.11
GIR (μmol · min^{-1} · kg FFM^{-1})	−0.33	0.04
Fasting total cholesterol (mmol/l)	0.22	0.17
Fasting HDL cholesterol (mmol/l)	−0.20	0.21
Fasting triglycerides (mmol/l)	0.39	0.01
Fasting NEFAs (mmol/l)	0.17	0.30
Adiponectin (μg/ml)	−0.34	0.03
C1q (mg/l)	0.34	0.03
C4 (mg/l)	0.30	0.06
C3 (g/l)	0.41	0.009
Factor B (mg/l)	0.43	0.005
Factor D (μg/ml)	0.10	0.56

*n = 36; †following 75-g oral glucose tolerance test. NEFA, nonesterified fatty acid.

tive complement pathway. No correlation was observed with factor D (which is not an acute-phase protein) (Table 2). Of the classical pathway components, only C1q showed a significant relationship with CRP (r = 0.34, P = 0.03).

CONCLUSIONS — The main findings of this study are as follows. *1*) The young, healthy, nonobese, normoglycemic, normolipidemic REL group had significantly lower insulin sensitivity than the CON group, who were similar for age, sex, and BMI. *2*) Despite 20% lower insulin sensitivity than CON subjects, REL subjects had normal and comparable levels of CRP and no evidence of complement consumption or acute-phase stimulation. *3*) CRP was not significantly related to insulin sensitivity independently of adiposity when all subjects were examined as a whole. *4*) CRP was significantly associated with acute-phase proteins of the alternative complement pathway.

Our results suggest that "inflammation" is not associated with early insulin resistance in nonobese first-degree relatives of subjects with type 2 diabetes. Although recent studies have led to speculation that CRP and the "inflammatory" response may induce insulin resistance (12), there is no direct evidence in humans. Furthermore, the notion that type 2 diabetes is an inflammatory disease is largely based on reports stating that increases (within the normal range) in the markers of inflammation, including CRP, white blood cell count, fibrinogen, orosomucoid, and sialic acid, predict its development (14–16,25). Traditionally, inflammatory disease involves elevation (i.e., above the normal range) of at least some of these markers, most consistently CRP, although their biological diversity suggests that not all, if any, would be directly involved in the inflammatory process. Moreover, in complement-related mechanisms, complement-derived enzymatic activation would be expected to lead to a net reduction in native complement levels. Neither of these findings were detected in the REL group. Hence, it seems inappropriate to use the term inflammation in the context of insulin resistance in the absence of significant elevation of CRP and consumption of complement.

Although the finding that CRP levels were not significantly different in nonobese REL and CON subjects concordant for adiposity but discordant for insulin sensitivity appears inconsistent with other reports, which, unlike our study, have specifically included obese subjects. In obese women participating in a weight-loss study, baseline CRP levels were higher in insulin-resistant than -sensitive subjects (based on steady-state plasma glucose levels during a modified insulin suppression test) matched for adiposity estimated by BMI and waist circumference (26). However, as the authors acknowledge, such surrogate estimates are a major limitation, concealing true differences in body fatness between the groups. In another study using bioelectrical impedance analysis, although obese insulin-resistant women (determined by the minimal model approach) had higher CRP levels than obese insulin-sensitive women matched for body fatness, paradoxically and contrary to their hypothesis, the obese insulin-sensitive group had significantly higher CRP levels than lean control subjects, despite similar insulin sensitivity (27). Importantly, and in contrast to these previous reports, REL subjects in the current study were young, healthy, normal-weight subjects whose only detectable metabolic abnormality was lower insulin sensitivity than CON subjects. The relationship between CRP and metabolic factors may be different in more extreme obesity and later in the evolution of type 2 diabetes. Thus, apparently discordant findings between studies may be explicable. At the very least, it appears inappropriate to implicate CRP and other factors as etiological agents in early disease states in younger subjects from the study of very obese subjects with more advanced disease.

Although the primary source of CRP is the liver, CRP mRNA has recently been identified in human subcutaneous adipose tissue (19). Furthermore, 25% of circulating interleukin-6 (IL-6), the principal stimulus of hepatic CRP production, is derived from adipocytes (28) or, as has been recently suggested, from macrophages within adipose tissue (29). While many studies strongly relate basal CRP levels to adiposity, CRP is also asso-

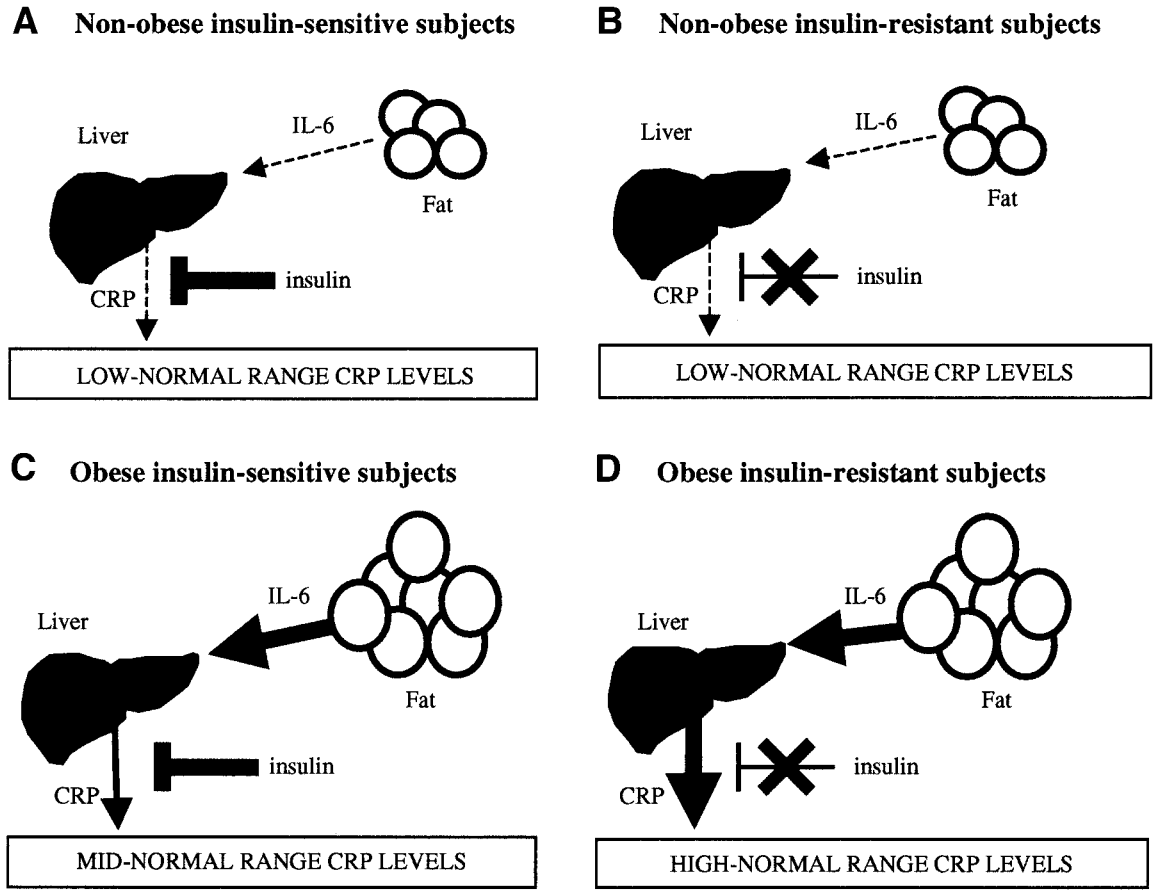

Figure 1—*Proposed interaction between primary upregulation of circulating CRP in proportion to body fatness and the inhibiting effect of insulin on IL-6–stimulated CRP secretion in nonobese insulin-sensitive subjects* (A)*, nonobese early insulin-resistant subjects (e.g., young first-degree relatives of subjects with type 2 diabetes)* (B)*, obese insulin-sensitive subjects* (C)*, and obese insulin-resistant subjects* (D)*. The thickness of the arrows relates to the amount of IL-6 or CRP secreted from fat and liver respectively. The thickness of the T-bar indicates the effectiveness of insulin in suppressing hepatic CRP production. In* A*, which applies to CON subjects, there is a relatively low-level stimulation of hepatic CRP production by IL-6, as the subjects are nonobese; hence, circulating levels of CRP are in the low-normal range. In* B*, which applies to REL subjects, although insulin inhibition of IL-6–mediated hepatic CRP production is impaired, there is only low-level stimulation from body fat; therefore, CRP levels are also in the low-normal range. In* C*, the tendency for CRP levels to be upregulated in proportion to body fatness is partially attenuated by insulin. In* D*, inhibition of CRP production by insulin is impaired, resulting in high-normal range CRP levels, in proportion to body fatness.*

ciated with insulin sensitivity (30–33). The confounding effect of body fat on the relationship between CRP and insulin sensitivity in our study confirms some (31,34), but not all (35,36), previous reports and suggests that the association between CRP and insulin sensitivity is not independent of adiposity. While differences in cohort size and characteristics may explain some disparity between studies, imprecision in the methodology for measuring adiposity and insulin sensitivity in many studies is also likely to be responsible. Conclusions about the role of adiposity cannot be made based on imprecise measures.

The lack of an independent relationship between CRP and insulin sensitivity in our study is also supported by recent studies investigating the relationship between changes in insulin sensitivity and changes in CRP levels. In one, there was no relationship between improvement in insulin sensitivity and change in CRP levels following 16 weeks of treatment with insulin or sulfonylureas (37). In another, pioglitazone-induced reductions in CRP levels in type 2 diabetic patients were independent of changes in glycemic control and insulin resistance (38). In a weight-loss study, the decrease in CRP levels after gastric surgery was related to the decrease in BMI but not to the change in insulin sensitivity (39).

It has recently been reported that CRP predicts the development of type 2 diabetes (14–16), with some groups reporting sex differences (40,41). Our results raise the possibility that body fat is the major determinant of this association and are consistent with some, but not all, previous studies (14,41) in which adjustment for BMI (among other factors) abolished the significant prediction of type 2 diabetes by CRP (15,16,42). Consistent with this hypothesis, in a study of Pima Indians, baseline CRP levels were not predictive of type 2 diabetes in 71 pairs of case and control subjects with similar BMI, waist circumference, age, and sex (22). A recent report suggests that this relationship may even be independent of both adiposity and insulin resistance, albeit estimated again by surrogate markers (14). Definitive conclusions regarding CRP and adiposity predicting type 2 diabetes cannot be made from studies using self-

reported anthropometric measurements (43).

Interestingly, across all subjects we found that CRP levels (within the normal range) were strongly positively correlated with levels of acute-phase complement proteins of the alternative pathway (i.e., C3 and factor B) but not factor D, which does not exhibit acute-phase reactivity. This low-grade acute-phase phenomenon may be an intrinsic ongoing protective property of the innate immune system (13), upregulated in proportion to body fatness. It is possible that this interaction is mediated by receptors reportedly involved in the inflammatory response, such as the toll receptors (44), although the ligand(s) that initiate the inflammatory cascade in obesity remain unknown. Reports that CRP has a heritability of 40–50% (45) and the identification of polymorphisms in the CRP gene (46) suggest that this intrinsic mechanism is largely genetically determined. Our finding that CRP was also weakly related to C1q, which may initiate classical pathway activation after CRP binding (17), may be consistent with the suggestion that C1q has a "cell-debris clearance" role (47). Interestingly, C1q has high homology to adiponectin (48), which was similar in the REL and CON groups and inversely related to CRP levels in our study. The latter finding supports recent reports and is consistent with the possibility that adiponectin modulates circulating CRP levels (19,22).

Insulin suppresses the levels of inflammatory markers. This has led some authors to suggest that insulin has anti-inflammatory properties (49), a proposal supported by evidence that it attenuates cytokine stimulation of acute-phase protein gene expression (50). Adipocyte-derived cytokines (such as IL-6) were not measured in this study. However, our data raise the possibility that in obese insulin-sensitive subjects, upregulation of circulating levels of acute-phase reactants in proportion to body fat remains under physiological regulation by insulin (Fig. 1). In contrast, in obese insulin-resistant subjects, the inhibition of these markers by insulin may be impaired (12), leading to "high-normal" circulating levels of CRP. If so, consistent with previous suggestions (25), rather than inflammation causing insulin resistance, marginal elevations of acute-phase proteins (within the normal range) are more likely to be a consequence of insulin resistance (Fig. 1). It has similarly been suggested that insulin resistance increases expression of proatherogenic endothelial adhesion molecules such as E-selectin (51). Regardless of the mechanisms involved, failure to suppress inflammation and endothelial activation in insulin resistance may in turn contribute to endothelial dysfunction and the development of atherosclerosis.

A major strength of this study was the use of two groups of young, healthy, nonobese subjects with normal glucose tolerance, differing only by the presence or absence of a family history of type 2 diabetes. This study design is essential to examine the early concomitants of insulin resistance per se, such as CRP, adiponectin, and complement protein levels, in the absence of potentially confounding factors such as hyperglycemia and hyperlipidemia. Our use of direct and gold standard measures of both insulin sensitivity and body fat was an additional major strength of the study. Although our results are biologically plausible, causality cannot be inferred given the cross-sectional nature of the study. Furthermore, our sample size may have limited our ability to detect small differences in inflammatory markers and complement proteins between CON and REL subjects. As subjects were recruited from advertisements, selection bias cannot be definitely excluded.

In conclusion, we found that nonobese relatives of subjects with type 2 diabetes were already less insulin sensitive than subjects with no family history of type 2 diabetes. However, these groups of subjects, who were similar for adiposity, also had similar levels of CRP, adiponectin, and complement proteins. This suggests that in subjects at high risk of type 2 diabetes, the insulin-resistant state per se is not characterized by inflammation. Importantly, our study confirms a strong relationship between CRP and body fat and suggests that the inverse association between CRP and insulin sensitivity is not independent of adiposity.

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