Complement Factor H Is Expressed in Adipose Tissue in Association With Insulin Resistance

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OBJECTIVE—Activation of the alternative pathway of the complement system, in which factor H (fH; complement factor H [CFH]) is a key regulatory component, has been suggested as a link between obesity and metabolic disorders. Our objective was to study the associations between circulating and adipose tissue gene expressions of CFH and complement factor B (fB; CFB) with obesity and insulin resistance.

RESEARCH DESIGN AND METHODS—Circulating fH and fB were determined by enzyme-linked immunosorbent assay in 398 subjects. CFH and CFB gene expressions were evaluated in 76 adipose tissue samples, in isolated adipocytes, and in stromovascular cells (SVC) (n = 13). The effects of weight loss and rosiglitazone were investigated in independent cohorts.

RESULTS—Both circulating fH and fB were associated positively with BMI, waist circumference, triglycerides, and inflammatory parameters and negatively with insulin sensitivity and HDL cholesterol. For the first time, CFH gene expression was detected in human adipose tissue (significantly increased in subcutaneous compared with omental fat). CFH gene expression in omental fat was significantly associated with insulin resistance. In contrast, CFB gene expression was significantly increased in omental fat but also in association with fasting glucose and triglycerides. The SVC fraction was responsible for these differences, although isolated adipocytes also expressed fB and fH at low levels. Both weight loss and rosiglitazone led to significantly decreased circulating fB and fH levels.

CONCLUSIONS—Increased circulating fH and fB concentrations in subjects with altered glucose tolerance could reflect increased SVC-induced activation of the alternative pathway of complement in omental adipose tissue linked to insulin resistance and metabolic disturbances. Diabetes 59:200–209, 2010

Obesity is closely associated with a cluster of metabolic diseases, such as dyslipidemia, hypertension, insulin resistance, type 2 diabetes, and atherosclerosis (1). Adipose tissue is well known for its essential role as an energy storage depot and for secreting adipokines that influence sites as diverse as brain, liver, muscle, β-cells, gonads, lymphoid organs, and systemic vasculature (2,3). Expression analysis of macrophage and nonmacrophage cell populations isolated from adipose tissue demonstrates that adipose tissue macrophages are responsible for most of the proinflammatory cytokines (4). In recent years, it has become evident that alterations in the function of the innate immune system are intrinsically linked to metabolic pathways in humans (5–8).

The complement system is a major component of the innate immune system, defending the host against pathogens, coordinating various events during inflammation, and bridging innate and adaptive immune responses. Complement deficiency and abnormalities in the regulation of the complement system lead to increased susceptibility to infection and chronic inflammatory diseases (9,10,11).

Factor H (fH) is a relatively abundant plasma glycoprotein that is essential to maintain complement homeostasis and to restrict the action of complement to activating surfaces. fH acts as a cofactor for factor I–mediated cleavage of C3b (the active fragment of the third component of complement C3), accelerates the dissociation of the alternative pathway C3 convertases (a bimolecular enzymatic complex formed by active fragments of C3 and factor B [fB]), and competes with fB for binding to C3b. fH regulates complement both in fluid phase and on cellular surfaces (12–16).

It has been suggested that activation of the alternative pathway of the complement system could be a link between obesity and metabolic disorders (17–21). Moreover, fB and factor D (fD, adipsin) are produced by adipose tissue where they likely influence formation of the alternative pathway component C3 convertase and the production of the anaphylatoxin C3a and its carboxypeptidase B-anaphylatoxic–inactivated derivative C3adesArg (acylating-stimulating protein [ASP]). Both ASP/C3adesArg and C3a interact with the receptor C5L2 to effectively stimulate triglyceride synthesis in cultured adipocytes (22). C3 knockout (C3KO) mice are obligatorily ASP deficient and present lipid abnormalities (23). In humans, ASP levels are increased in obesity, type 2 diabetes, and in individuals at risk of arterial disease, including those with hypertension, type 2 diabetes, dyslipidemia, and coronary artery disease, whereas exercise or weight loss decreases ASP levels (24,25). These data suggest a relationship between these conditions and activation of the alternative pathway of...
complement. There is also a correlation between increased C3 concentration and decreased insulin action (26,27). Levels of C3 and fB were higher in subjects with insulin resistance and other features of the metabolic syndrome (28,29).

Given these interactions among activation of the alternative pathway of complement, metabolic disturbances, and a chronic low-level inflammatory state, we designed experiments to study the associations among circulating fH, fB, insulin resistance, lipid parameters, and inflammatory markers. We found that circulating fH and fB are strongly associated with obesity. For this reason, we also studied whether adipose tissue could constitute a source of circulating fH and fB.

RESEARCH DESIGN AND METHODS

For this study 338 Caucasian men were recruited, and 259 subjects were randomly localized from a census and were invited to participate. The participation rate was 71%. A 75-g oral glucose tolerance test (OGTT), according to the American Diabetes Association criteria, was performed in all subjects. All subjects with normal glucose tolerance (NGT) (n = 140) had fasting plasma glucose <7.0 mmol/l and 2-h postload plasma glucose <7.5 mmol/l after a 75-g OGTT. Glucose intake and insulin concentrations in the glucose disappearance according to the American Diabetes Association Criteria (postload glucose 7.5–11.1 mmol/l). Previously unknown type 2 diabetes was diagnosed in 36 additional subjects (postload glucose >11.1 mmol/l). Subjects with glucose intolerance and type 2 diabetes were grouped as altered glucose tolerance (AGT).

Inclusion criteria were 1) BMI <40 kg/m², 2) absence of systemic disease, and 3) absence of infection within the previous month. None of the control subjects were under medication or had evidence of metabolic disease other than obesity. Liver disease and thyroid dysfunction were specifically excluded by biochemical workup.

In order to increase the statistical power of the group of patients with type 2 diabetes, 139 patients were prospectively recruited from diabetes outpatient clinics on the basis of stable metabolic control in the previous 6 months, defined by stable A1C values. Data from these patients were merged with those from the recently diagnosed type 2 diabetic patients. Exclusion criteria for these patients included the following: 1) clinically significant hepatic, neurological, endocrinologic, or other major systemic disease including malignancy; 2) history or current clinical evidence of hemochromatosis; 3) history of drug or alcohol abuse, defined as >80 g/day in men and >40 g/day in women; 4) elevated serum creatinine concentration; 5) acute major cardiovascular event (including previous myocardial infarction <6 months); 6) evidence of acute or chronic inflammatory or infective diseases; and 7) mental illness rendering the subjects unable to understand the nature, scope, and possible consequences of the study. Pharmacological treatment for these patients was insulin, 31 patients; metformin, 37 patients; sulfonylureas, 16 patients; statins, 34 patients; fibrates, 9 patients; blood pressure–lowering agents, 28 patients; and angiotensin-converting enzyme, 9 patients. All subjects gave written informed consent after the purpose of the study was explained to them. The institutional review board approved the protocol.

Subjects were studied after at least 10 h of fasting. BMI was calculated as weight in kilograms divided by height in meters squared. Blood pressure was measured in the supine position on the right arm after a 10-min rest; a standard sphygmomanometer of appropriate cuff size was used, and the first and fifth phases were recorded. Values used in the analysis are the average of three readings taken at 5-min intervals.

Insulin sensitivity was measured using the frequently sampled intravenous glucose tolerance test on a different day in those subjects who agreed (n = 147). In brief, basal blood samples were drawn at 0, 15 and 5 min, after which glucose (300 mg/kg body wt) was injected over 1 min starting at time 0. At 20 min, regular insulin (0.03 units/kg actrapid; Novo Nordisk, Denmark) was injected as a bolus. Additional samples were obtained from a contralateral antecubital vein at times 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 19, 20, 22, 23, 24, 25, 27, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, and 180 min. Samples were rapidly collected via a three-way stopcock connected to a butterfly needle. Data from the frequently sampled intravenous glucose tolerance test were submitted to computer programs that calculate the characteristic metabolic parameters by fitting glucose and insulin to the minimal model that describes glucose disappearance, provides the parameters for the insulin sensitivity index (10⁻⁴ min⁻¹ µU⁻¹ · ml⁻¹), or a measure of the effect of insulin concentrations above the basal level to enhance glucose disappearance. The estimation of model parameters was performed according to the minimal model (MINMOD) analysis computer program (30).

Insulin resistance was also measured by the homeostasis model assessment of insulin resistance (HOMA-IR). HOMA-IR correlates well with insulin sensitivity derived from the glucose clamp technique (r = −0.82; P < 0.0001) (31). In the weight loss study, an indirect measure of insulin sensitivity was calculated from the fasting plasma glucose and insulin concentrations by using the quantitative insulin sensitivity check index (QUICKI) (32).

Complement fH and fB expression in adipose tissue, stromal vascular fraction, and isolated adipocytes. A group of 76 adipose tissue samples (38 omental and 38 subcutaneous depots) from participants (19 men and 19 women, aged 43.7 ± 9.9 years, mean BMI 43.7 ± 8.6 kg/m², fasting glucose 110.6 ± 28.9 mg/dl, log fasting insulin 1.12 ± 0.36 mU/l, total cholesterol 200.1 ± 40.77 mg/dl, HDL cholesterol 46.2 ± 18.5 mg/dl, LDL cholesterol 128.7 ± 33.3 mg/dl, and log fasting triglycerides 2 ± 0.2 mg/dl) who were recruited at the Endocrinology Department at the University Clinic of Navarra (Pamplona, Spain) and at the Endocrinology Service of the Hospital Univer- sitario de Granada (Josep Trueta) (Granada, Spain) were of the recently diagnosed type 2 diabetic Caucasian and reported that their body weight had been stable for ≥3 months before the study. Liver and renal diseases were specifically excluded by biochemical workup. All subjects gave written informed consent after the purpose of the study was explained to them.

Adipose tissue samples were obtained from subcutaneous and omental depots during elective surgical procedures (cholecystectomy, surgery of abdominal hernia, and gastric bypass surgery). Both subcutaneous and omental fat were obtained from the abdomen following standard procedures.

To analyze adipose tissue gene expression, tissues were washed, fragmented, and immediately flash frozen in liquid nitrogen before being stored at −80°C.

To perform the isolation of adipocyte and stromal vascular fraction (SVF), tissues were washed three to four times with PBS and suspended in an equal volume of PBS supplemented with 1% bovine serum albumin (BSA) and 0.1% collagenase (Agilent Technologies, Palo Alto, CA). Total RNA was quantified by means of spectrophotometer (GeneQuant, GE Health Care, Piscataway NJ) and immediately flash frozen in liquid nitrogen before being stored at −80°C. To perform the isolation of adipocyte and stromal vascular fraction (SVF), tissues were washed three to four times with PBS and suspended in an equal volume of PBS supplemented with 1% bovine serum albumin (BSA) and 0.1% collagenase (Agilent Technologies, Palo Alto, CA). Total RNA was quantified by means of spectrophotometer (GeneQuant, GE Health Care, Piscataway NJ) and immediately flash frozen in liquid nitrogen before being stored at −80°C.
Sequence Detection System (Applied Biosystems, Darmstadt, Germany), using TaqMan technology suitable for relative genetic expression quantification.

The commercially available and prevalidated TaqMan primer/probe sets used were as follows: endogenous control PPIA (4333763, cyclophilin A) and target genes complement fH (CFH) (Hs00164830_m1) and complement fB (CFB) (Hs00156060_m1). The RT-PCR TaqMan reaction was performed in a final volume of 25 μl. The cycle program consisted of an initial denaturing of 10 min at 95°C then 40 cycles of 15-s denaturizing phase at 95°C and a 1-min annealing and extension phase at 60°C. A threshold cycle (Ct value) was obtained for each amplification curve, and a ΔCt value was first calculated by subtracting the Ct value for human Cyclophilin A (PPIA) RNA from the Ct value for each sample. Fold changes compared with the endogenous control were then determined by calculating 2−ΔΔCt, so gene expression results are expressed as an expression ratio relative to PPIA gene expression according to manufacturers’ guidelines.

**Study of the effects of weight loss.** We recruited 42 Caucasian obese volunteers (22 women, 20 men, and age range 27–70 years) attending the Endocrinology Department at the University Clinic of Navarra. Patients underwent a clinical assessment including medical history, physical examination, body composition analysis, and comorbidity evaluation as well as nutritional interviews performed by a multidisciplinary consultation team. All subjects were nonsmokers. Patients with signs of infection were excluded. Obese patients were not receiving statins or antidiabetes medication.

Weight loss was achieved by prescription of a diet providing a daily energy deficit of 500–1,000 kcal/day calculated from the determination of the resting energy expenditure through indirect calorimetry (Vmax29; SensorMedics, Yorba Linda, CA) and multiplication by 1.4, indicated for sedentary individuals to obtain the patients’ total energy expenditure during 4 months. This hypocaloric regimen allows a safe and steady weight loss of 0.5–1.0 kg/week when followed and supplied 30, 54, and 16% of energy requirements in the form of fat, carbohydrates, and protein, respectively. In this study body weight was measured with a digital scale to the nearest 0.1 kg, and height was measured to the nearest 0.1 cm with a Holtain stadiometer (Holtain, Crymych, U.K.). The institutional review board of the participant institutions approved the protocol, so we certify that all applicable institutional regulations concerning the ethical use of information and samples from human volunteers were followed during this research.

**Effects of rosiglitazone in circulating fB and fH.** We conducted a parallel-group randomized and controlled trial to evaluate the effects of rosiglitazone on endothelial function in patients with coronary artery disease and pre-diabetes over a 6-month period. Consecutive patients of either sex (5 women, 15 men, and age range 37–72 years) referred to the University of Leipzig Heart Center, Germany, were invited to participate if they displayed impaired fasting glucose (≥6.0 and ≤7.0 mmol/l with A1C <6%) or impaired glucose tolerance (≥7.8 and <11.1 mmol/l 2 h after oral intake of 75 g glucose) and angiographic evidence of coronary artery disease (>50% stenosis diameter in at least one major epicardial artery).

Exclusion criteria included diabetes type 1 or 2, preexisting antidiabetes medication, unstable angina, indication for coronary bypass surgery, significant left main disease, myocardial infarction within preceding 3 months, ejection fraction <40%, significant heart valve disease, severe metabolic disorders, severe disorders in lipoprotein metabolism, thyroid disorders, alcohol or drug abuse, pregnancy, and participation in another trial.

Eligible patients were randomly assigned to one of two treatment groups...
for 6 months: group A received 8 mg rosiglitazone daily (Avandia; GlaxoSmithKline, London, U.K.) (n = 12), group B served as a control group (n = 8). Patients who served as control subjects did not receive rosiglitazone. Randomization was performed by drawing sealed opaque envelopes. At day 1 (baseline) after 4 weeks and 6 months, patients were clinically examined, endothelial function was assessed, and blood samples were obtained. The protocol was approved by the local ethics committee. All subjects gave written informed consent before enrollment.

**Analytical methods.** Serum glucose concentrations were measured in duplicate by the glucose oxidase method using a Beckman Glucose Analyzer II (Beckman Instruments, Brea, CA). AIC was measured by the high-performance liquid chromatography method (Bio-Rad, Muenchen, Germany, and autoanalyzer Jokoh HS-10, respectively). Intra-assay and interassay coefficients of variation (CVs) were <1% for all tests. HDL cholesterol was quantified after precipitation with polyethylene glycol at room temperature. Total serum triglycerides were measured through the reaction of glycerol phosphate oxidase and peroxidase. Serum insulin was measured in duplicate in the same centralized laboratory by a monoclonal immunoradiometric assay (Medgenix Diagnostics, Fleunes, Belgium). The intra-assay CV was 5.2% at a concentration of 10 mU/l and 3.4% at 130 mU/l. The interassay CVs were 6.9 and 4.5% at 14 and 89 mU/l, respectively.

Serum-soluble tumor necrosis factor-α receptor-2 (sTNFR2) concentration was measured by sTNFR-II EASIAM ELISA kit (Biosource Europe, Belgium); serum lipopolysaccharide binding protein (LBP) levels were measured by a human LBP EASIAM kit (HyCult biotechnology, PB Uden, the Netherlands). Intra- and interassay coefficients of variation for all these determinations were between 5–10%.

**Complement ELISA.** fH and fB levels were measured by a sandwich ELISA method. In brief, 96-well microtiter plates were coated overnight with a polyclonal rabbit α-human fH or fB antibodies (capture antibody) diluted in 0.1 mol/l NaHCO3 pH 8.5 buffer at 4°C. After blocking for 1 h at room temperature with 50 mmol/l Tris pH 7.4, 150 mmol/l NaCl 0.2% Tween 20, and 1% BSA, samples were added and incubated for 1 h at room temperature. Appropriate dilutions of purified human fH and fB were used to prepare a standard curve. Two in-house mouse monoclonals α-human fH (35F9) and α-human fB (JCI) were used as detecting antibodies. These monoclonal antibodies are well-characterized reagents that have been used extensively in ELISA sandwich immuno-chromatography methodology to measure levels of fH and fB and to purify these proteins from human plasma (33,34). After incubation with rabbit α-mouse Ig antibodies conjugated to horseradish peroxidase, (DAKO, Denmark) plates were developed with o-phenyl-diamine (DAKO, Denmark) and absorbance measured at 492 nm. Intra- and interassay CVs for all these determinations were 2–8%.

**Statistical methods.** Statistical analyses were performed using SPSS 12.0 software. Unless otherwise stated, descriptive results of continuous variables are expressed as means ± SD for Gaussian variables. Parameters that did not fulfill normal distribution were logarithmically transformed to improve symmetry for subsequent analyses. The relation between variables was analyzed by simple correlation (Pearson test) and multiple regression analyses. Unpaired and paired t tests were used to compare NGT and AGT subjects and the effects of weight loss or rosiglitazone and the comparison of fH and fB gene expression between subcutaneous and omental adipose tissue, respectively. Levels of statistical significance were set at P < 0.05.

**RESULTS**

**Circulating fB and fH concentration analysis.** The metabolic characteristics of both groups are shown in Table 1. Circulating fH concentrations were significantly associated with circulating fB concentrations (r = 0.65; P < 0.0001). Circulating fH and fB were significantly increased in subjects with AGT (195.4 ± 63.5 vs. 175.2 ± 53.4 μg/ml, P = 0.01) and 285.9 ± 90.5 vs. 231.95 ± 58.8, P < 0.0001, respectively) (Table 1). Concomitant treatment in subjects with type 2 diabetes did not influence circulating fH and fB levels significantly.

In all subjects, both circulating fH and fB were positively associated with BMI, waist-to-hip ratio, systolic and diastolic blood pressure, fasting glucose, GHb, log fasting triglycerides, LBP, and sTNFR2 and negatively with log insulin sensitivity index and HDL cholesterol (Fig. 1, Table 2).

| TABLE 3 | Correlation between CPT concentrations and study variables in the cross-sectional study |
|---------|-----------------------------------------------------------------------------------------|
| n       | r                                          | p  |
| All subjects | CPH (μg/ml) | GGT | 0.38 | 0.1  |
| AGT     | CPH (μg/ml) | GGT | 0.43 | 0.1  |
| 258     | 0.68 | 0.01 |

**Table 3**

| TABLE 4 | Insulin sensitivity index* |
|---------|---------------------------|
| n       | 147                       |
| P       | < 0.0001                  |

| *Insulin sensitivity was measured in 147 subjects (83 subjects with NGT and 64 subjects with AGT) using the frequently sampled intravenous glucose tolerance test. Significant data are shown in bold.
In AGT subjects, these associations were strengthened. In addition, the associations of circulating fH and fB with log fasting triglycerides (r = 0.30, P < 0.0001; and r = 0.30, P < 0.0001, respectively), LBP (r = 0.26, P = 0.001; and r = 0.52, P < 0.0001, respectively), log insulin sensitivity index (r = −0.40, P = 0.001; and r = −0.42, P = 0.001, respectively), and HDL cholesterol (r = −0.23, P = 0.005; and r = −0.33, P < 0.0001, respectively) were especially significant (Table 2).

In all subjects, age (P = 0.03) and log insulin sensitivity index (P = 0.01) contributed independently to 20.4% of circulating fB variance after controlling for the effects of waist-to-hip ratio, log fasting triglycerides, and LBP in multiple linear regression models. Only log insulin sensitivity index (P = 0.02) contributed independently to 12.2% of circulating fH variance in the same multiple linear regression model.

Circulating fH and fB concentration in treated type 2 diabetic patients was not significantly different versus untreated type 2 diabetic patients (212.9 ± 65.3 vs. 212.2 ± 79.2 μg/ml, P = 0.9; and 327.4 ± 96.48 vs. 311.4 ± 102.2 μg/ml, P = 0.4, respectively). The possible contribution of each treatment in the prediction of fH and fB variance was analyzed using multiple linear regression models. None of the treatments had significant effects on fH or fB concentrations.

**Complement fH and fB expression analysis in adipose tissue.** fH gene expression was significantly increased in subcutaneous compared with omental adipose tissue (0.57 ± 0.22 vs. 0.40 ± 0.2 relative units, P = 0.004).

### Table 3
Correlation between omental and subcutaneous CFB and CFH expression and selected metabolic parameters

|                        | Omental (n = 38) | Subcutaneous (n = 38) |
|------------------------|-----------------|----------------------|
|                        | CFB expression  | CFH expression       | CFB expression | CFH expression |
|                        | r    | P    | r   | P    | r    | P    | r   | P    |
| Age (years)            | −0.07 | 0.7  | −0.12 | 0.5  | −0.25 | 0.17 | −0.49 | 0.005 |
| BMI (kg/m²)            | 0.35  | 0.05 | 0.23  | 0.18 | −0.02 | 0.9  | −0.01 | 0.3   |
| Fasting glucose (mg/dl)| 0.44  | 0.02 | 0.25  | 0.16 | −0.02 | 0.93 | −0.14 | 0.4   |
| Fasting insulin (mU/l) | 0.11  | 0.5  | 0.50  | 0.004 | 0.03  | 0.85 | 0.22  | 0.25  |
| HOMA-IR                | 0.13  | 0.5  | 0.49  | 0.006 | 0.08  | 0.66 | 0.15  | 0.4   |
| Fasting triglycerides (mg/dl) | 0.48  | 0.02 | 0.02  | 0.9  | −0.04 | 0.87 | −0.2  | 0.37  |
| HDL cholesterol (mg/dl)| −0.63 | 0.002 | −0.34 | 0.1  | −0.07 | 0.7  | 0.02  | 0.9   |

Significant data are shown in bold.

**FIG. 2.** Study of CFH and CFB relative gene expression in SVC and adipocytes from omental (OM) and subcutaneous (SC) fat depots.

Insulin sensitivity index were observed (Table 2). In AGT subjects, these associations were strengthened. In addition, the associations of circulating fH and fB with log fasting triglycerides (r = 0.30, P < 0.0001; and r = 0.30, P < 0.0001, respectively), LBP (r = 0.26, P = 0.001; and r = 0.52, P < 0.0001, respectively), log insulin sensitivity index (r = −0.40, P = 0.001; and r = −0.42, P = 0.001, respectively), and HDL cholesterol (r = −0.23, P = 0.005; and r = −0.33, P < 0.0001, respectively) were especially significant (Table 2).

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**Complement fH and fB expression analysis in adipocytes and SVF cells.** fH and fB were expressed in both cell fractions from omental and subcutaneous fat depots. Again, fB gene expression was significantly higher in omental than subcutaneous fat depot but specifically in cells from the SVC fraction (0.096 ± 0.03 vs. 0.022 ± 0.006 relative units, P = 0.001). Complement CFH gene expression was increased significantly in cells from the SVC fraction of the subcutaneous fat depot than in cells from the SVC fraction of the omental fat depot (0.40 ± 0.19 vs. 0.24 ± 0.02 relative units, P = 0.04). In both fat depots, fH gene expression was significantly higher than complement fB gene expression (Fig. 2). No differences between fat depots were found regarding fH and fB gene expression in adipocytes (Fig. 2).

**Weight loss study.** Characteristics of the subjects are shown in Table 5. In this independent cohort, the associ-
Baseline and post-weight loss subject characteristics in the weight loss study.

|         | Baseline | Post-weight loss | P     |
|---------|----------|------------------|-------|
| n       | 42       | 42               |       |
| Age (years) | 43.1 ± 15 | 43.1 ± 15       |       |
| BMI (kg/m²) | 36.3 ± 8.4 | 30.3 ± 5.3     | <0.0001 |
| Waist-to-hip ratio | 0.95 ± 0.08 | 0.93 ± 0.07 | 0.01  |
| Systolic blood pressure (mmHg) | 126.2 ± 15.9 | 119.7 ± 12.7 | 0.015 |
| Diastolic blood pressure (mmHg) | 80.6 ± 9.6 | 75.7 ± 6.8 | 0.003 |
| Fasting glucose (mg/dl) | 95.5 ± 10.7 | 90.1 ± 7.9 | 0.008 |
| Insulin (mU/l) | 17.9 ± 13.8 | 11.7 ± 6.1 | 0.04  |
| Total cholesterol (mg/dl) | 204.4 ± 35.8 | 175.6 ± 25.3 | <0.001 |
| HDL cholesterol (mg/dl) | 53.4 ± 12.4 | 51.5 ± 11.7 | 0.23  |
| LDL cholesterol (mg/dl) | 129.02 ± 32.9 | 107.9 ± 22.9 | <0.0001 |
| Log 10 fasting triglycerides (mg/dl) | 2.04 ± 1.6 | 1.9 ± 1.48 | <0.0001 |
| QUICKI | 0.32 ± 0.03 | 0.35 ± 0.04 | 0.01  |
| HOMA-IR | 4.2 ± 3.4  | 2.56 ± 1.4  | 0.04  |
| fH (µg/ml) | 264.7 ± 59.3 | 235.15 ± 72 | 0.009 |
| fB (µg/ml) | 304.26 ± 75.5 | 274.3 ± 67 | 0.025 |

Data are means ± SD unless otherwise indicated.

Correlations of circulating fB and fH concentrations with BMI, waist circumference, and insulin sensitivity (QUICKI index) were replicated (Fig. 3). Circulating fB and fH concentrations also were associated with fasting triglycerides ($r = 0.33$, $P = 0.04$ for both). Weight loss led to a significant decrease of circulating fB and fH concentrations (Fig. 4). Interestingly, the decrease in circulating fB and fH concentrations was mainly observed in young subjects, defined as younger than the median age of the cohort (42 years) (Fig. 4). The baseline ratio of fB to fH was associated with weight loss: the higher the ratio, the higher the weight loss ($r = 0.41; P = 0.008$). Again, this association was mainly observed in young subjects (31 ± 8.06 years) ($r = 0.61; P = 0.006$) but not in older subjects (56.3 ± 7.7 years) ($r = 0.23; P = 0.3$).

In agreement with this age-discordant effects, the decrease in fasting triglycerides concentrations was parallel to that of fB and fH in older subjects ($r = 0.48, P = 0.02$; and $r = 0.44, P = 0.04$, respectively) but not in younger subjects ($r = -0.26, P = 0.2$; and $r = -0.31, P = 0.3$, respectively). The decrease of fH and fB after weight loss was similar in men and women (fH in men: from 279.2 ± 61.6 to 249.4 ± 75.5 relative units, $P = 0.06$ and fB in men: from 302.5 ± 80.4 to 276.1 ± 60.5 relative units, $P = 0.1$; fH in women: from 248.6 ± 53.5 to 219.4 ± 66.2 relative units, $P = 0.07$ and fB in women: from 306.1 ± 71.8 to 272.3 ± 71.1 relative units, $P = 0.1$).

**Effects of rosiglitazone administration.** Subjects treated with rosiglitazone and control subjects were similar in sex (10 men and 2 women vs. 5 men and 3 women, $P = 0.35$), age (65 ± 7.7 years vs. 65.5 ± 11.7 years, $P = 0.9$), and BMI (30.2 ± 4.2 vs. 31.3 ± 4.3 kg/m², $P = 0.5$). Rosiglitazone led to significantly increased insulin sensitivity (glucose infusion rate during the clamp: from 46 ± 9.5 to

FIG. 3. Baseline linear relationships between CFH and CFB with BMI, waist circumference, and insulin sensitivity (QUICKI) in the weight loss study.
67.4 ± 9.2 µmol · kg⁻¹ · min⁻¹, P < 0.0001), whereas BMI did not change significantly (30.8 ± 4.3 kg/m², P = 0.1). In control subjects, glucose infusion rate tended to decrease (from 46.9 ± 10.9 to 44.9 ± 9.4 vs. µmol · kg⁻¹ · min⁻¹, P = 0.05), whereas BMI remained unchanged (31.5 ± 4.5, kg/m², P = 0.3).

Circulating fH concentration decreased significantly (by −35%; P = 0.02) in parallel to increased insulin sensitivity in subjects receiving rosiglitazone (Fig. 5B). In control subjects, circulating fH concentration tended to increase (from 202.7 ± 32.1 to 228.3 ± 28.8 µg/ml, P = 0.09). Interestingly, the change in glucose infusion rate was inversely associated with the change of circulating fH concentrations (r = −0.49; P = 0.02) (Fig. 5A). Circulating fB did not change significantly in either group (264.9 ± 62.6 vs. 270.3 ± 64.7 µg/ml, P = 0.5 in subjects receiving rosiglitazone; 265.3 ± 53.9 vs. 272.7 ± 57.1 µg/ml, P = 0.7 in control subjects).
alternative pathway of complement has been previously reported in individuals at risk of arterial disease linked to central fatness, including those with hypertension, type 2 diabetes, dyslipidemia, and coronary artery disease (18,21,22). fH might function as a buffer mechanism to counteract the activation of the alternative complement pathway associated with increased expression of fB and fD in obesity and insulin resistance. However, the increased fH expression in adipose tissue could be caused by a global deregulation of adipocyte-derived production of proinflammatory mediators. In addition, adipocyte-relevant functions of these proteins are not currently known.

The physiological role of the adipose tissue is to act as an energy store, accumulating triglycerides in times of plenty for use in times of need. Adipocytes express all the components required for the local generation of C3a and ASP/C3adesArg, which can then act to mediate this storage function. Both C3a and ASP/C3adesArg have potent activity as acylation-stimulating proteins, promoting the esterification of fatty acids into triglycerides, raising membrane transport of glucose into adipocytes, and increasing the activity of diacylglycerol acyltransferase. All these effects markedly increase the rate of triglyceride synthesis (39). fD expression is increased in fasting or catabolic states and decreased in various models of obesity. It is therefore possible that modulating its capacity to activate the complement alternative pathway, the adipocyte helps to adjust its physiology to the need for triglyceride storage or release. We observed a cross-sectional association between fasting triglycerides, fB, and fH concentrations in two different cohorts. After weight loss, the decrease in the circulating concentrations of all these factors run in parallel but only in those subjects over the median age of the cohort. Mean fB and fH concentrations did not change significantly after weight loss in this latter group, suggesting that the relationship between these complement factors and triglycerides is not modifiable once a critical age is reached. However, this hypothesis should be explored more in depth.

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pathway is known to lead to severe inflammatory diseases (14,40,41), including partial lypodistrophy. This is a rare disease in which there is loss of fat usually from the upper part of the body. The disease is frequently associated with mesangiocapillary (membranoproliferative) glomerulonephritis type II, unbalanced local production of fB and fD, and deficiencies in fH (42,43). Interestingly, we observed that an increased baseline fB-to-fH ratio was directly associated with the degree of weight loss in young subjects. Again, this observation needs to be replicated in a larger series of subjects. Further functional studies are necessary to test this hypothesis.

Complement is an essential part of innate immunity and plays a central role in the elimination of microbes, clearing of immune complexes and damaged self cells, and modulating the adaptive immune response. Complement genes are regulated by cytokines, such as interleukin-1, interleukin-6, tumor necrosis factor-α (TNF-α), or interferon-γ at the transcriptional level (44). The chronic inflammatory state associated with insulin resistance and obesity could lead to an increased gene expression and activation of the complement pathway. In this study, circulating fH and fB were significantly and positively associated with inflammatory parameters (sTNFR2 and LBP). sTNFR2 is a known surrogate of endogenous TNF-α action. LBP is produced by the liver in response to bacterial endotoxin and constitutes an endotoxin marker (45). High-fat diet has been recently described to raise endotoxin translocation from the gut into the bloodstream, leading to endotoxin-induced metabolic alterations named as metabolic endotoxemia (46,47). Current results suggest that metabolic endotoxemia could stimulate alternative complement activation in omental adipose tissue.

We here confirmed the expression of fB in adipose tissue (18,21). Its association with insulin resistance has been less studied. Again, circulating fB concentration, as a marker of alternative complement pathway activation, was significantly associated with insulin resistance and several metabolic parameters (blood pressure, fasting triglycerides, and HDL cholesterol, mainly in AGT subjects; Table 2). Adipose tissue CFB gene expression tended to be higher in subjects with insulin resistance (Table 3B), and omental CFB gene expression was significantly associated with fasting glucose and triglycerides (positively) and negatively with HDL cholesterol (Table 3A).

The expression of fB was increased in omental compared with subcutaneous adipose tissue, and the reverse was found regarding fH. The increased expression of CFB and CFB genes in adipose tissue was observed in the SVF cells (composed by monocytes, fibroblasts, and endothelial cells). In addition, significant gene expressions of these factors were also found in mature adipocytes, although without differences between fat depots. In conclusion, increased circulating fB and fH concentration in subjects with AGT could reflect the elevated SVC-induced alternative complement pathway activation in omental adipose tissue linked to insulin resistance and metabolic disturbances. Further studies are necessary to evaluate the role of fH in metabolism.

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J.M.F.-R. had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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