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; CFH) is a key regulatory component, has been suggested as a link between obesity and metabolic disorders. 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 ELISA in 398 subjects. CFH and CFB gene expressions were evaluated in 76 adipose tissue samples, in isolated adipocytes and stroma-vascular cells (SVC) (n=13). The effects of weight loss and rosiglitazone were investigated in independent cohorts.

**Results:** Both circulating fH and fB were positively associated with BMI, waist diameter, 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 the responsible of 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.
Obesity is closely associated with a cluster of metabolic diseases, such as dyslipidaemia, hypertension, insulin resistance, type 2 diabetes and atherosclerosis (1). Adipose tissue is well known for its essential role as energy storage depot and for secreting adipokines which influence sites as diverse as brain, liver, muscle, β cells, gonads, lymphoid organs, and systemic vasculature (2,3). Expression analysis of macrophage and non-macrophage cell populations isolated from adipose tissue demonstrates that adipose tissue macrophages are responsible for most of the pro-inflammatory 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. Factor H 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. Factor H 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 C3 convertase and the production of the anaphylatoxin C3a and its carboxypeptidase B-anaphylatoxic-inactivated derivative C3adesArg (ASP; acylation-stimulating-protein). Both ASP/C3adesArg and C3a interact with the receptor C5L2 to effectively stimulate TG 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 in Pima Indians (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 between 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 that reason, we also studied whether adipose tissue could constitute a source of circulating fH and fB.

METHODS

Patient recruitment. Three hundred ninety eight Caucasian men were recruited and studied. Two hundred fifty nine subjects were randomly
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localized from a census and they were invited to participate. The participation rate was 71%. A 75g 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 mM and two-hour post-load plasma glucose < 7.8 mM after a 75g OGTT. Glucose intolerance was diagnosed in 83 subjects according to the American Diabetes Association Criteria (post-load glucose between 7.8 and 11.1 mM). Previously unknown type 2 diabetes was diagnosed in 36 additional subjects (post-load glucose higher than 11.1 mM/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 work-up.

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 a stable metabolic control in the previous 6 months, as defined by stable HbA1c 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 creatinin concentration; 5) acute major cardiovascular event in the previous 6 months; 6) acute illnesses and current 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: 38 patients; aspirin: 42 patients; and allopurinol: 3 patients. All subjects gave written informed consent after the purpose of the study was explained to them. The institutional review board of the institution approved the protocol.

**Measurements.** Subjects were studied after at least 10 hours of fasting. BMI was calculated as weight (in kg) divided by height (in m) 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.** Insulin sensitivity was measured using the frequently sampled intravenous glucose tolerance test (FSIVGTT) on a different day in those subjects who agreed (n=147). In brief, basal blood samples were drawn at –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 (Actrapid, Novo, Denmark; 0.03 U/kg) 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 FSIVGTT were submitted to computer programs that calculate the characteristic metabolic parameters by fitting glucose and insulin to the minimal model that describes the times course of glucose and insulin concentrations. The glucose disappearance model, by accounting for the effect of insulin and glucose on glucose disappearance, provides the parameters $S_i (10^{-4})$ per minute per microunit per milliliter) or the insulin sensitivity index, 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 MINMOD 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
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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 CFH and CFB expression in adipose tissue, stromal vascular fraction (SVF) and in 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 43.9 ± 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, 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 Universitari Dr. Josep Trueta (Girona, Spain), were analyzed. All subjects were of Caucasian origin and reported that their body weight had been stable for at least three months before the study. Liver and renal diseases were specifically excluded by biochemical work-up. 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 by-pass surgery). Both subcutaneous and omental fat were obtained from the abdomen, following standard procedures. To analyse adipose tissue gene expression, tissues were washed, fragmented and immediately flash-frozen in liquid nitrogen before stored at -80°C. To perform the isolation of adipocyte and SVF, tissues were washed three to four times with phosphate-buffered saline (PBS) and suspended in an equal volume of PBS supplemented with 1% bovine serum and 0.1% collagenase type I prewarmed to 37°C. The tissue was placed in a shaking water bath at 37°C with continuous agitation for 60 minutes and centrifuged for 5 minutes at 300 to 500g at room temperature. The supernatant, containing mature adipocytes, was recollected. The pellet was identified as the SVF cell. The adipose tissue fractionation was performed from 7 omental and 6 subcutaneous depots.

RNA was prepared from these samples using RNeasy Lipid Tissue Mini Kit (Qiagen, US). The integrity of each RNA sample was checked by Agilent Bioanalyzer (Agilent Technologies, Palo Alto, CA). Total RNA was quantified by means of spectrophotometer (GeneQuant, GE Health Care, Piscataway NJ) reverse transcribed to cDNA using High Capacity cDNA Archive Kit (Applied Biosystems, Darmstadt, Germany) according to the manufacturer’s protocol.

Gene expression was assessed by real time PCR using an ABI Prism 7000 Sequence Detection System (Applied Biosystems, Darmstadt, Germany), using TaqMan® technology suitable for relative genetic expression quantification. The commercially available and pre-validated TaqMan® primer/probe sets used were as follows: endogenous control PPIA (4333763, cyclophilin A) and target genes CFH (Hs00164830_m1, Complement Factor H), CFB (Hs00156060_m1, Complement Factor B). The RT-PCR TaqMan® reaction was performed in a final volume of 25µl. The cycle program consisted of an initial denaturing of 10min at 95°C then 40 cycles of 15sec denaturizing phase at 95°C and 1min 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
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compared with the endogenous control were then determined by calculating $2^{\Delta Ct}$, so gene expression results are expressed as expression ratio relative to PPIA gene expression according to manufacturers’ guidelines.

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

Weight loss was achieved by prescription of a diet providing a daily energy deficit of 500-1000 kcal/d as calculated from the determination of the resting energy expenditure through indirect calorimetry (Vmax29, SensorMedics Corporation, Yorba Linda, California) and multiplication by 1.4 as indicated for sedentary individual’s to obtain the patient’s total energy expenditure during four months. This hypocaloric regime allows a safe and steady weight loss of 0.5-1.0 kg/wk 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 Ltd., Crymych, UK).

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 prediabetes over a 6-month period. Consecutive patients of either sex (5 women, 15 men, 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 HbA1c <6%) or impaired glucose tolerance (>7.8 and <11.1 mmol/L 2 hours after oral intake of 75 grams of glucose) and angiographic evidence of coronary artery disease (>50% stenosis diameter in at least one major epicardial artery).

Exclusion criteria included diabetes mellitus type 1 or 2, pre-existing antidiabetic 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 2 treatment groups for 6 months: Group A received rosiglitazone (Avandia, GlaxoSmithKline, London, United Kingdom) 8 mg daily (n=12), group B served as a control group (n=8). Patients who served as controls received neither 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. ClinicalTrials.gov Identifier NCT00298909.
Analytical methods. Serum glucose concentrations were measured in duplicate by the glucose oxidase method using a Beckman glucose analyser II (Beckman Instruments, Brea, California). HbA1c was measured by the high-performance liquid chromatography method (Bio-Rad, Muenchen, Germany, and autoanalyser Jokoh HS-10, respectively). Intraassay and interassay coefficients of variation were less than 4% for all these 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 inter-assay 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 sTNF-RII EASIATM ELISA kit (Biosource Europe S.A., Belgium); Serum lipopolysaccharide binding protein (LBP) levels were measured by Human LBP ELISA kit (HyCult biotechnology b.v.; PB Uden, The Netherlands). Intra- and interassay coefficients of variation for all these determinations were between 5-10%.

Complement ELISA. Factor H 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 0.1M NaHCO3 pH9.5 buffer at 4°C. After blocking for 1 hour at room temperature with 50mM Tris pH 7.4, 150mM NaCl 0.2% Tween 20 and 1% BSA, samples were added and incubated for 1 hour 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 (35H9) and α-human fB (JC1) were used as detecting antibodies. These monoclonal antibodies are well characterized reagents that have been used extensively in ELISA and affinity-chromatography methodologies to measure levels of fH and fB and to purify these proteins from human plasma (33,34). After incubation with a rabbit α-mouse Ig antibodies conjugated to HRP (DAKO, Denmark) plates were developed with o-phenyl-diamine (DAKO, Denmark) and absorbance measured at 492nm.

Intra- and interassay coefficients of variation for all these determinations were between 2-8%.

Statistical methods. Statistical analyses were performed using SPSS 12.0 software. Unless otherwise stated, descriptive results of continuous variables are expressed as mean and 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’s 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
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circulating fB and fH concentrations (Figure 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) (Figure 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=0.4, respectively), but not in younger subjects (r=-0.26, p=0.2, r=-0.31, p=0.3, respectively).

The decrease of fH and fB following weight loss was similar in men and women (fH, from 279.2 ± 61.6 to 249.4 ± 75.5 (p=0.06); and fB, from 302.5 ± 80.4 to 276.1 ± 60.5 (p=0.1) in men; and fB, from 248.6 ± 53.5 to 219.4 ± 66.2 (p=0.07); and fB, from 306.1 ± 71.8 to 272.3 ± 71.1 (p=0.1), in women).

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 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 67.4 ± 9.2 µmol/kg/min, p<0.0001) while 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 ± 9.4 vs. µmol/kg/min, p=0.05) while 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 (Figure 5-B). 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) (Figure 5-A).

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).

DISCUSSION
Plasma complement proteins are mainly derived from the liver, although other tissues can contribute significantly (35). The main components of the classical and alternative complement pathways are also produced in the adipose tissue (18-20). In 1989, adipsin, a major protein product of mature adipocytes, was reported to be highly homologous to factor D, a serine protease whose only known function was the cleavage of factor B in the Complement alternative pathway (36).

Adipsin and factor D were later demonstrated to be identical, with adipose tissue being a major source of factor D in vivo, and adipocytes also producing C3 and factor B and activating the alternative pathway in vitro and in vivo (20, 37). Subsequently, another link between the adipocyte and the complement system was apparent with the description of adiponectin, an adipocyte-specific product which is highly homologous to C1q (38). To the best of our knowledge, this is the first report demonstrating CFH gene expression in human adipose tissue and circulating fH levels in association with obesity measures. CFH gene expression has been reported in mice adipose tissue and non-differentiated adipocytes, but not in association with obesity or insulin resistance (37).
Both adipose tissue $CFH$ gene expression and circulating $fH$ concentration were significantly associated with insulin resistance and several metabolic parameters (blood pressure, lipid parameters) mainly in AGT subjects. Interestingly, the association between $CFH$ gene expression and insulin resistance was only found in the omental fat depot. Improvement of insulin resistance induced by weight loss or rosiglitazone led to decreased circulating $fH$ concentrations. The unchanged BMI of subjects treated with rosiglitazone, and the relationship between the change in insulin sensitivity and circulating $fH$ levels suggest that insulin resistance is a driving force in the induction of increased $fH$ levels. In fact, alterations in innate immunity are well known in obesity and insulin resistance. Activation of the 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 component 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, rising 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 not only a cross-sectional association between fasting triglycerides, $fB$ and $fH$ concentrations in 2 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. A failure in the regulation of the alternative complement 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, and unbalanced local production of $fB$, $fD$ and deficiencies in $fH$ (42,43). Interestingly, we observed that an increased baseline $fB/fH$ ratio was directly associated with the degree of weight loss in young subjects. Again, this observation needs to be replicated in 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 gamma 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 endotoxemia 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, were 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 were 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 CFH and CFB genes in adipose tissue was observed in the stromal vascular fraction 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 fH and fB 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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José Manuel Fernández-Real 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.
REFERENCES
Table 1. Clinical characteristics of subjects in the cross-sectional study

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<th>AGT Mean ± SD</th>
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<td>BMI (Kg/m²)</td>
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<td>28.9 ± 5.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>WHR</td>
<td>0.92 ± 0.06</td>
<td>0.97 ± 0.07</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>122.9 ± 14.6</td>
<td>139.8 ± 20.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>77.9 ± 10.7</td>
<td>81.8 ± 10.2</td>
<td>0.001</td>
</tr>
<tr>
<td>Fasting Glucose (mg/dl)</td>
<td>92.15 ± 8.05</td>
<td>160.3 ± 82.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>4.7 ± 0.32</td>
<td>7.01 ± 1.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>204.05 ± 35</td>
<td>205.5 ± 37.9</td>
<td>0.7</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>53.8 ± 13.02</td>
<td>48.3 ± 12.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>132.5 ± 31.6</td>
<td>120.2 ± 38.9</td>
<td>0.002</td>
</tr>
<tr>
<td>Log 10 Fasting Triglycerides (mg/dl)</td>
<td>1.9 ± 0.24</td>
<td>2.2 ± 0.27</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>sTNFR2 (ng/ml)</td>
<td>6.49 ± 3.5</td>
<td>8.02 ± 5.25</td>
<td>0.001</td>
</tr>
<tr>
<td>LBP (µg/ml)</td>
<td>19.7 ± 13.9</td>
<td>44.2 ± 29.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Insulin sensitivity *</td>
<td>0.58 ± 0.2</td>
<td>0.34 ± 0.18</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Complement factor H (µg/ml)</td>
<td>175.2 ± 53.4</td>
<td>195.4 ± 63.5</td>
<td>0.01</td>
</tr>
<tr>
<td>Complement factor B (µg/ml)</td>
<td>231.95 ± 58.8</td>
<td>285.9 ± 90.5</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

NGT, normal glucose tolerance; AGT, altered glucose tolerance; BMI, Body Mass Index; WHR, Waist to hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, glycated hemoglobin; sTNFR2, soluble tumor necrosis factor receptor 2; LBP, Lipopolysaccharide Binding Protein; *Insulin sensitivity was measured in 147 subjects (83 subjects with NTG and 64 subjects with AGT) using the frequently sampled intravenous glucose tolerance test.
Table 2. Correlation between Complement Factor H concentrations and study variables in the cross-sectional study

<table>
<thead>
<tr>
<th></th>
<th>Complement Factor H (µg/ml)</th>
<th>Complement Factor B (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All subjects</td>
<td>NGT</td>
</tr>
<tr>
<td><strong>N</strong></td>
<td>398</td>
<td>140</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>0.22</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>WHR</td>
<td>0.27</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>0.21</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>0.15</td>
<td>0.004</td>
</tr>
<tr>
<td>Fasting Glucose (mg/dl)</td>
<td>0.12</td>
<td>0.03</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>0.04</td>
<td>0.4</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>0.01</td>
<td>0.9</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>0.23</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>0.08</td>
<td>0.1</td>
</tr>
<tr>
<td>Log Fasting Triglycerides (mg/dl)</td>
<td>0.30</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>sTNFR2 (ng/ml)</td>
<td>0.12</td>
<td>0.03</td>
</tr>
<tr>
<td>LBP (µg/ml)</td>
<td>0.25</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Insulin sensitivity*</td>
<td>0.26</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>fH (µg/ml)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

NGT, normal glucose tolerance; AGT, altered glucose tolerance; BMI, Body Mass Index; WHR, Waist to hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, glycated hemoglobin; sTNFR2, soluble tumor necrosis factor receptor 2; LBP, Lipopolysaccharide Binding Protein; *Insulin sensitivity was measured in 147 subjects (83 subjects with NTG and 64 subjects with AGT) using the frequently sampled intravenous glucose tolerance test.
Table 3. Correlation between omental and subcutaneous CFB and CFH expression and selected metabolic parameters.

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

Table 4. Complement factor expression according to insulin resistance status.

<table>
<thead>
<tr>
<th></th>
<th>Subjects with HOMA value &lt; 3.9 (n=20) Mean ± SD</th>
<th>Subjects with HOMA value &gt; 3.9 (n=18) Mean ± SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omental CFH expression</td>
<td>0.35 ± 0.16</td>
<td>0.59 ± 0.12</td>
<td>0.003</td>
</tr>
<tr>
<td>Omental CFB expression</td>
<td>0.11 ± 0.03</td>
<td>0.16 ± 0.04</td>
<td>0.08</td>
</tr>
<tr>
<td>Subcutaneous CFH expression</td>
<td>0.56 ± 0.19</td>
<td>0.57 ± 0.22</td>
<td>0.9</td>
</tr>
<tr>
<td>Subcutaneous CFB expression</td>
<td>0.0137 ± 0.08</td>
<td>0.0136 ± 0.06</td>
<td>0.9</td>
</tr>
</tbody>
</table>
Table 5. Subjects’ characteristics in the weight loss study.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Post-weight loss</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participants</td>
<td>42</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>43.1 ± 15</td>
<td>43.1 ± 15</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>36.3 ± 8.4</td>
<td>30.3 ± 5.3</td>
<td></td>
</tr>
<tr>
<td>WHR</td>
<td>0.95 ± 0.08</td>
<td>0.93 ± 0.07</td>
<td>0.01</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>126.2 ± 15.9</td>
<td>119.7 ± 12.7</td>
<td>0.015</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>80.6 ± 9.6</td>
<td>75.7 ± 6.8</td>
<td>0.003</td>
</tr>
<tr>
<td>Fasting Glucose (mg/dl)</td>
<td>95.5 ± 10.7</td>
<td>90.1 ± 7.9</td>
<td>0.008</td>
</tr>
<tr>
<td>Insulin (mU/l)</td>
<td>17.9 ± 13.8</td>
<td>11.7 ± 6.1</td>
<td>0.04</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>204.4 ± 35.8</td>
<td>175.6 ± 25.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>53.4 ± 12.4</td>
<td>51.5 ± 11.7</td>
<td>0.23</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>129.02 ± 32.9</td>
<td>107.9 ± 22.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Log 10 Fasting Triglycerides (mg/dl)</td>
<td>2.04 ± 1.6</td>
<td>1.9 ± 1.48</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.32 ± 0.03</td>
<td>0.35 ± 0.04</td>
<td>0.01</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>4.2 ± 3.4</td>
<td>2.56 ± 1.4</td>
<td>0.04</td>
</tr>
<tr>
<td>fH (µg/ml)</td>
<td>264.7 ± 59.3</td>
<td>235.15 ± 72</td>
<td>0.009</td>
</tr>
<tr>
<td>fB (µg/ml)</td>
<td>304.26 ± 75.5</td>
<td>274.3 ± 67</td>
<td>0.025</td>
</tr>
</tbody>
</table>

Results are presented as mean ± SD; BMI: Body Mass Index; WHR: Waist-to-hip ratio; SBP: systolic blood pressure; DBP: diastolic blood pressure; QUICKI, Insulin sensitivity index; HOMA-IR: homeostasis model assessment of insulin resistance.

Figure legends

**Figure 1.** Linear relationships between complement factor H, complement factor B and body mass index and log insulin sensitivity index (derived from the minimal model).

**Figure 2.** Study of complement factor H (CFH) and complement factor B (CFB) relative gene expression in SVC and adipocytes from omental and subcutaneous fat depots.

**Figure 3.** Baseline linear relationships between complement factor H, complement factor B and body mass index, waist circumference and insulin sensitivity (QUICKI) in the weight loss study.

**Figure 4.** Changes in circulating complement factor H and complement factor B after weight loss in all subjects (left panels) and according to age (right panels). Subjects were classified into younger and older than 42 years (median age of the cohort).

**Figure 5.** a. Linear relationship between the change in circulating Factor H (%) and the change in glucose infusion rate during the clamp (%) (r=-0.49, p=0.02). b. 95% confidence interval for the mean of the percent change in circulating Factor H and glucose infusion rate following rosiglitazone and in control subjects.
Complement Factor H and insulin resistance.

**Figure 1**

- **First graph:**
  - Correlation coefficient: $r = 0.22$, $p < 0.0001$, $n = 398$
  - BMI (kg/m²) vs. Complement Factor H (µg/ml)

- **Second graph:**
  - Correlation coefficient: $r = -0.26$, $p < 0.0001$, $n = 147$
  - Log insulin sensitivity index vs. Complement Factor H (µg/ml)

- **Third graph:**
  - Correlation coefficient: $r = 0.21$, $p < 0.0001$, $n = 398$
  - BMI (kg/m²) vs. Complement Factor B (µg/ml)

- **Fourth graph:**
  - Correlation coefficient: $r = -0.27$, $p < 0.0001$, $n = 147$
  - Log insulin sensitivity index vs. Complement Factor B (µg/ml)
Complement Factor H and insulin resistance.

Figure 2

![Graph showing relative expression levels with p-values 0.04 and 0.001]

Relative expression

<table>
<thead>
<tr>
<th>CFH OM</th>
<th>CFH SC</th>
<th>CFB OM</th>
<th>CFB SC</th>
<th>CFH OM</th>
<th>CFH SC</th>
<th>CFB OM</th>
<th>CFB SC</th>
</tr>
</thead>
<tbody>
<tr>
<td>SVC</td>
<td>Adipocyte</td>
<td>SVC</td>
<td>Adipocyte</td>
<td>SVC</td>
<td>Adipocyte</td>
<td>SVC</td>
<td>Adipocyte</td>
</tr>
</tbody>
</table>
Figure 3
Figure 4

(a) Complement Factor H (ug/ml) at baseline and post-weight loss.

(b) Complement Factor B (ug/ml) at baseline and post-weight loss.

Complement Factor H and insulin resistance.
**Figure 5**

**a**

$r = -0.49, \ p = 0.02$

**b**

$p = 0.02$

$p < 0.0001$