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WISP1 Is a Novel Adipokine Linked to Inflammation in Obesity

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WISP1 (Wnt1-inducible signaling pathway protein-1, also known as CCN4) is a member of the secreted extracellular matrix–associated proteins of the CCN family and a target gene of the Wingless-type (WNT) signaling pathway. Growing evidence links the WNT signaling pathway to the regulation of adipogenesis and low-grade inflammation in obesity. We aimed to validate WISP1 as a novel adipokine. Human adipocyte differentiation was associated with increased WISP1 expression and secretion. Stimulation of human macrophages with WISP1 led to a proinflammatory response. Circulating WISP1 and *WISP1* subcutaneous adipose tissue expression were regulated by weight changes in humans and mice. WISP1 expression in visceral and subcutaneous fat tissue was associated with markers of insulin resistance and inflammation in glucose-tolerant subjects. In patients with nonalcoholic fatty liver disease, we found no correlation among disease activity score, liver fat content, and *WISP1* expression. Insulin regulated *WISP1* expression in adipocytes in vitro but had no acute effect on WISP1 gene expression in subcutaneous fat tissue in overweight subjects who had undergone hyperinsulinemic clamp experiments. The data suggest that WISP1 may play a role in linking obesity to inflammation and insulin resistance and could be a novel therapeutic target for obesity.

The obesity epidemic is a growing health, social, and economic problem worldwide (1). Central obesity and metabolic syndrome are independent risk factors for type 2 diabetes, cancer, nonalcoholic fatty liver disease (NAFLD), and an insulin-resistant state (1,2). Over the past decade, a unifying mechanism behind the pathogenesis of obesity-associated diseases has given birth to the concept of "meta-inflammation," which describes the chronic low-grade inflammatory response to obesity (3). The limited expandability of adipose tissue is another determinant in the pathogenesis of obesity-associated diseases (4). Furthermore, abundant evidence, mostly derived from mouse studies, links the Wingless-type (WNT) signaling pathway to the regulation of adipogenesis (5,6) and inflammation (7) in obesity.

WNT signaling family members are secreted glycoproteins that act in both autocrine and paracrine fashions to regulate cell proliferation, cell fate, differentiation, and organism development (8,9). The WNT signaling network comprises multiple so-called "canonical" and "noncanonical" pathways that lead to tightly controlled cell remodeling. WNT-inducible signaling pathway protein-1 (WISP1, also known as CCN4) belongs to the CCN family of extracellular matrix proteins and is a downstream target gene

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of the canonical WNT signaling pathway (10). Experimental evidence suggests that other CCN family members, such as WISP2 and nephroblastoma overexpressed protein (NOV), participate in the pathogenesis of obesity and associated diseases (5,6,11,12), but no data are available on the possible relationship between WISP1 and obesity. Similar to the majority of CCN proteins, WISP1 consists of four globular modules analogous to various extracellular protein domains. Module I is homologous to the IGF-binding domain. Module II is a von Willebrand factor type C repeat module, and module III represents thrombospondin type I, which is important for cell attachment. Module IV consists of a COOH-terminal domain (13). WISP1 is expressed in various organs and tissues, including the heart, pancreas, lung, kidney, small intestine, ovaries, spleen, and brain, and in some of these tissues, acts antiapoptotically through the phosphatidylinositol 3-kinase (PI3K) and Akt pathways (14). WISP1 serves a regulatory function in skeletal growth and bone repair (15). It regulates mesenchymal proliferation and osteoblastic differentiation as well as chondrogenic differentiation (16). Moreover, WISP1 is upregulated in a variety of cancers (17) and has been associated with invasion of cholangiocarcinoma (18). Thus, WISP1 can determine the onset and progression of apoptosis and autophagy during normal physiology, acute illness, or chronic degenerative disorders (14).

No data are currently available regarding the effects of WISP1 on insulin target tissues, including liver and fat. In the current study, we combined in vitro experiments with four independent clinical studies to validate WISP1 as a novel adipokine and to characterize the association of WISP1 with parameters of the metabolic syndrome. We show that *1*) WISP1 is a novel adipokine released from differentiated human adipocytes; *2*) WISP1 expression is substantially elevated in visceral adipose tissue (VAT) rather than in subcutaneous adipose tissue (SAT) in glucose-tolerant subjects; *3*) *WISP1* expression correlates with insulin sensitivity, adiponectin, and markers of adipose tissue inflammation; *4*) weight reduction decreases *WISP1* expression in SAT as well as circulating WISP1 levels in plasma; and *5*) hepatic *WISP1* expression shows no association with ectopic fat accumulation in obesity.

RESEARCH DESIGN AND METHODS

Cohort I

Paired samples of VAT and SAT were obtained from 75 Caucasian men (n = 40) and women (n = 35) who underwent abdominal surgery and were metabolically characterized as described previously (19). Percentage body fat was measured by DEXA. In a subgroup (n = 52), abdominal visceral fat content was measured by magnetic resonance imaging (MRI) as described previously (20). Insulin sensitivity was assessed with the euglycemic-hyperinsulinemic clamp method as described previously (21). Macrophage content in VAT and SAT samples was visualized in adipose tissue sections (stained with hematoxylin-eosin) by additional staining against CD68 (1:200; DAKO) as described previously (19). One hundred cells were studied from each slide, and $CD68^+$ cells were counted. Adipocyte cell size in VAT and SAT were analyzed in adipose tissue sections as described previously (19).

Cohort II

Effects of weight reduction were studied in 49 subjects kept on an 8-week low-calorie diet (Modifast; Nutrition et Santé, Revel, France) consisting of 800 kcal/day plus 200 g/day vegetables. Participants who achieved a weight loss of at least 8% after 8 weeks were selected for the study. A detailed description of the study design has been previously published (22). Percentage of body fat was measured by DEXA. Plasma samples and SAT biopsy specimens were collected after overnight fasting before and after weight loss.

Cohort III

In the human liver study, 47 patients met the inclusion criteria, gave informed consent, and were enrolled as described previously (23). Comparable to liver tissue, perfused samples of VAT and abdominal SAT were harvested by knife extraction before therapeutic interventions. All samples were flash frozen in liquid nitrogen and stored at −80°C until the RNA extraction procedure. A part of the liver sample was used for histopathological analysis after fixing in 4% formalin (Histofix, Roth, Germany), embedding in paraffin, and staining with hematoxylin-eosin. The unweighted sum of points for the intensity of liver steatosis, lobular inflammation, fibrosis, and hepatocellular ballooning was used to calculate the NAFLD activity score (NAS) and to identify subjects with undefined and definite nonalcoholic steatohepatitis (NASH) (23). Study subjects were accordingly classified as NASH (NAS $\geq$3) or non-NASH (NAS $<$3). Exclusively healthy liver tissue was used for analyses because a blinded clinical expert pathologist considered all harvested liver samples to be histologically normal with respect to pathologies except NAFLD.

Cohort IV

Fourteen healthy, moderately obese male subjects were recruited to participate in one or two of the following procedures in a randomized design: *1*) control experiment (0.9% saline infusion) (n = 8); *2*) euglycemic-hyperinsulinemic clamp for 4 h with continuous infusion of 40 · mU · m^2 body surface · min^{-1} human insulin at a steady-state capillary plasma glucose concentration of 4.4 mmol/L (80 mg/dL) (n = 10); and *3*) hyperinsulinemic-hyperglycemic clamp for 4 h with continuous infusion of 40 · mU · m^2 body surface · min^{-1} human insulin at a steady-state capillary plasma glucose concentration of 7.8 mmol/L (140 mg/dL) (n = 8), as described previously (24). Venous blood samples for analysis of hormones were taken at −70, 0, +210, +230, and +240 min timed from the start of insulin infusion, and SAT biopsy specimens were taken before and after infusions (time = −40 min and +240 min).

In cohorts I and III, paired samples of VAT and SAT were obtained from the same sites during abdominal

surgery by knife extraction. In cohorts II and IV, SAT biopsy specimens were taken by needle aspiration from contralateral sites at the level of the umbilicus. All samples were immediately flash frozen in liquid nitrogen and stored at −80°C for mRNA extraction.

Study I was approved by the ethics committee of the University of Leipzig, Germany, and studies II–IV were approved by the ethics committees of *1*) Potsdam University, Potsdam, Germany; *2*) State Medical Association of Brandenburg, Germany; and *3*) Charité University Medicine, Berlin, Germany. All subjects gave written informed consent before taking part in the study.

Biochemical Measurements

Biochemical measurements were performed by routine methods. WISP1 in plasma and medium samples were detected by commercial assay (WISP1 ELISA; RayBiotech, Inc., Norcross, GA). For this, medium samples were concentrated using Vivaspin 2 concentrators (Sartorius, Goettingen, Germany). Cytokine release in cell culture medium was quantified using ProcartaPlex Multiplex Immunoassays (eBioscience, Frankfurt am Main, Germany).

Animal Study

Animal protocols were approved by the local governmental animal ethics review board (State of Brandenburg, Germany). Twelve-week-old male C57BL/6J mice were kept on either a control diet (10 kcal-% fat, 20 kcal-% protein, 70 kcal-% carbohydrate, 3.85 kcal/g) or a high-fat diet (HFD) (60 kcal-% fat, 20 kcal-% protein, 20 kcal-% carbohydrate, 5.24 kcal/g) (Research Diets, Inc., New Brunswick, NJ) for 6 weeks. *WISP1* mRNA expression was measured in epididymal white adipose tissue, liver, and gastrocnemius muscle.

Cell Culture

Human monocyte–derived macrophages were differentiated from isolated blood monocytes in RPMI 1640 medium supplemented with 10% HyClone FCS (Thermo Scientific, Waltham, MA) and 50 ng/mL human granulocyte macrophage colony-stimulating factor (Peprotech, Hamburg, Germany) for 7 days and stimulated with recombinant human WISP1 (*Escherichia coli* endotoxin <0.1 ng/μg protein; Peprotech) from 50 ng/mL to 1.5 μg/mL for 24 h.

Human mesenchymal stem cells were isolated from abdominal SAT samples (25) and differentiated into mature adipocytes in DMEM-F12 supplemented with 500 μmol/L 3-isobutyl-1-methylxanthine, 25 nmol/L dexamethasone, 0.2 nmol/L 3,3,5-triiodo-L-thyronine, 8 μg/mL D-biotin, 15 mmol/L D-pantothenate, 100 nmol/L hydrocortisone, 20 nmol/L insulin, 0.01 mg/mL transferrin (all from Sigma-Aldrich, Seelze, Germany), and 2 μmol/L rosiglitazone (Cayman Chemical, Ann Arbor, MI) for 14 days. Adipocyte morphology was confirmed by Oil Red O staining (Supplementary Fig. 1*A*). Differentiated adipocytes were treated with 100 nmol/L insulin (Sigma-Aldrich) for 4 h or with 0.5 μg/mL WISP1 (Peprotech) for 24 h.

3T3-L1 adipocytes were differentiated in DMEM supplemented with 10% HyClone FCS, 1 μg/L insulin, 0.5 mmol/L 3-isobutyl-1-methylxanthine, and 0.25 μmol/L dexamethasone for 7 days and treated with or without LY294002 (25 μmol/L, Sigma-Aldrich), PD098059 (30 μmol/L, Sigma-Aldrich), and NVP-AEW541 (0.1 μmol/L, Cayman Chemicals) 30 min before stimulation with 100 nmol/L insulin for 4 h.

Oil Red O Staining

Differentiated adipocytes were washed in PBS and fixed with 10% formaldehyde for 60 min at room temperature. Thereafter, each well was gently rinsed with water, and 60% isopropanol was added to each well for 5 min. Oil Red O working solution was prepared by mixing three parts Oil Red O stock solution (Sigma-Aldrich) with two parts deionized water. The cultures were incubated with Oil Red O working solution for 5 min at room temperature. Oil Red O solution was removed, and the cultures were washed with tap water until the rinses ran clear.

Quantitative Real-Time PCR

Total RNA was extracted by NucleoSpin RNA II Kit (Macherey-Nagel, Düren, Germany) or RNeasy Lipid Tissue Mini Kit (QIAGEN, Hilden, Germany). Synthesis of cDNA was performed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). Quantitative real-time PCR (qRT-PCR) was performed using Power SYBR Green PCR Master Mix (Applied Biosystems) and primers shown in Supplementary Table 1.

Western Blotting

Semidry immunoblots were performed with antibodies specific to phospho-p44/42 mitogen-activated protein kinase (MAPK) (phosphorylated extracellular signal–related kinase [ERK]), p44/42 MAPK (ERK), phospho-Akt and Akt (Life Technologies/Cell Signaling, Boston, MA), WISP1 (ab10737, Abcam, Cambridge, MA), α-tubulin (Life Technologies/Cell Signaling), and IRDye 800CW Goat anti-Rabbit IgM (LI-COR Biosciences, Lincoln, NE) and quantified by the Odyssey Infrared Imaging System (LI-COR Biosciences, Bad Homburg, Germany).

Statistical Analysis

SPSS version 20.0 software (IBM Corporation, Chicago, IL) was used for all statistical analyses. If not stated otherwise, data are mean ± SEM. Presence or absence of normal distribution was verified by the Kolmogorov-Smirnov test. Depending on the distribution of data, Pearson simple coefficient or Spearman rank correlation coefficient was used for correlation analysis, and Mann-Whitney *U* test or Student *t* test was applied to estimate differences between groups. $P < 0.05$ was considered significant.

RESULTS

WISP1 Gene Expression in SAT and VAT Is Associated With Markers of Insulin Sensitivity and Adipose Tissue Inflammation

Baseline characteristics of cohort I–IV are summarized in Table 1. *WISP1* mRNA expression in VAT was higher than

Table 1—Clinical characteristics of studied cohorts

	Cohort I	Cohort II[a]	Cohort III		Cohort IV
			Non-NASH (NAS <3)	NASH (NAS ≥3)	
n (% male)	75 (53)	49 (38)	34 (41)	13 (38)	14 (100)
T2D (*n*)	0	1	5	4	0
Age (years)	51.9 ± 1.6	40.4 ± 0.8	58 ± 3	62 ± 3	47.4 ± 2.2
BMI (kg/m^2)	24.0 ± 0.1	34.5 ± 0.6	24.2 ± 0.6	32.1 ± 2.1**	32.5 ± 0.6
Body fat (%)	20.8 ± 0.3	38.6 ± 1.4	ND	ND	31.7 ± 1.1
Fasting plasma glucose (mmol/L)	5.3 ± 0.1	5.2 ± 0.1	5.5 ± 0.3	6.4 ± 1.1	4.9 ± 0.09
Fasting plasma insulin (pmol/L)	41.0 ± 6.3	78.5 ± 20.1	50.3 ± 7.3	84.6 ± 16.8*	79.2 ± 11.9
HOMA-IR (mmol · mU/L^2)	1.59 ± 0.24	3.82 ± 1.06	2.08 ± 0.40	4.61 ± 1.34	2.97 ± 0.46
AST/ALT	ND	ND	1.44 ± 0.17	1.42 ± 0.33	ND
NAS (0–8)	ND	ND	0.94 ± 0.14	3.88 ± 0.38**	ND

Data are mean ± SEM unless otherwise indicated. Statistical differences were determined by Mann-Whitney *U* test. ALT, alanine transaminase; AST, aspartate transaminase; ND, not done; T2D, type 2 diabetes. [a]Only data before weight loss intervention are shown. *$P < 0.05$ vs. control subjects in cohort III. **$P < 0.01$ vs. control subjects in cohort III.

in SAT in normal glucose-tolerant subjects in cohort I (Fig. 1*A*). We hypothesized that the level of *WISP1* mRNA expression in adipose tissue is associated with established markers of obesity, insulin resistance, and inflammation. Indeed, *WISP1* mRNA levels correlated positively with fasting insulin ($r = 0.28$ [$P = 0.02$] and $r = 0.25$ [$P = 0.03$] for expression in VAT and SAT, respectively) and macrophage infiltration ($r = 0.52$ [$P = 0.03$] and $r = 0.66$ [$P = 0.003$] for expression in VAT and SAT, respectively) and negatively with insulin sensitivity, measured as glucose infusion rate in the euglycemic-hyperinsulinemic clamp experiment ($r = -0.31$ [$P = 0.008$] and $r = -0.32$ [$P = 0.006$] for expression in VAT and SAT, respectively) (Fig. 1*B–G*), as well as circulating adiponectin ($r = -0.24$ [$P = 0.046$] for expression in VAT; data not shown). The SAT expression of *WISP1* correlated with VAT expression ($r = 0.25$, $P = 0.02$). We observed a slight association with BMI ($r = 0.18$ [$P = 0.11$] and $r = 0.16$ [$P = 0.17$] for VAT and SAT, respectively), waist-to-hip ratio ($r = 0.17$ [$P = 0.15$] and $r = 0.20$ [$P = 0.08$] for VAT and SAT, respectively), and fat cell size in SAT ($r = 0.25$, $P = 0.048$) but not in VAT ($r = 0.12$, $P = 0.33$) and no correlation with total body fat content ($r = 0.08$ [$P = 0.48$] and $r = -0.17$ [$P = 0.14$] for expression in VAT and SAT, respectively). In the subgroup of cohort I ($n = 52$), we observed a positive correlation of *WISP1* expression in VAT with visceral fat content measured by MRI ($r = 0.23$, $P = 0.026$; data not shown).

In cell culture experiments, we found that *WISP1* mRNA was expressed in human mesenchymal stem cell–derived adipocytes, and its expression was further increased during adipocyte differentiation (Fig. 1*H* and Supplementary Fig. 1*A* and *B*). The increase of *WISP1* mRNA expression during adipocyte differentiation was accompanied by an elevation of intracellular WISP1 protein expression (Supplementary Fig. 1*C*) as well as of WISP1 release into the culture medium (Fig. 1*I*). *WISP1* expression was found neither in human monocytes nor in human monocyte-derived M and GM macrophages (data not shown).

We next stimulated human macrophages and human mesenchymal stem cell–derived adipocytes with WISP1 for 24 h. In the macrophage culture, we observed a significant and dose-dependent WISP1-induced increase of interleukin (IL)-6, tumor necrosis factor-α (TNFA), IL1B, and IL10 at the mRNA expression level as well as at the protein level in the culture medium (Figs. 2 and 3*A*). Moreover, WISP1 induced an increase in expression of inflammatory M1 markers of macrophage polarization CCR7 and COX2. In line with this, expression of M2 marker CD36 and, in tendency, CD163 was decreased (Fig. 3*B*). By contrast, we did not observe a WISP1-mediated response in adipocytes with regard to TNFA, IL1B, and IL10 either at the mRNA level or in the culture medium. Of note, the IL6 concentration in the culture medium of adipocytes was decreased after stimulation with WISP1 (Fig. 2*B*). We also found no influence of WISP1 on the adipocyte differentiation in vitro analyzed using Oil Red O staining (Supplementary Fig. 1*B*) and mRNA expression of adipocyte markers (data not shown). Moreover, we observed no effects of WISP1 on the activation of insulin signaling in adipocytes as measured by quantification of phosphorylated Akt and ERK protein levels (Supplementary Fig. 2).

WISP1 Is Inversely Regulated by HFD-Induced Obesity and Weight Loss

We next examined the expression of *WISP1* in the SAT of subjects who underwent weight loss with a low-calorie diet (19). We observed a reduction of *WISP1* mRNA expression in SAT after the weight loss ($P = 0.04$), whereas WISP1 level in plasma was not significantly changed in the general cohort (Fig. 4*A* and *B*). Nevertheless, basal *WISP1* mRNA expression in SAT strongly correlated

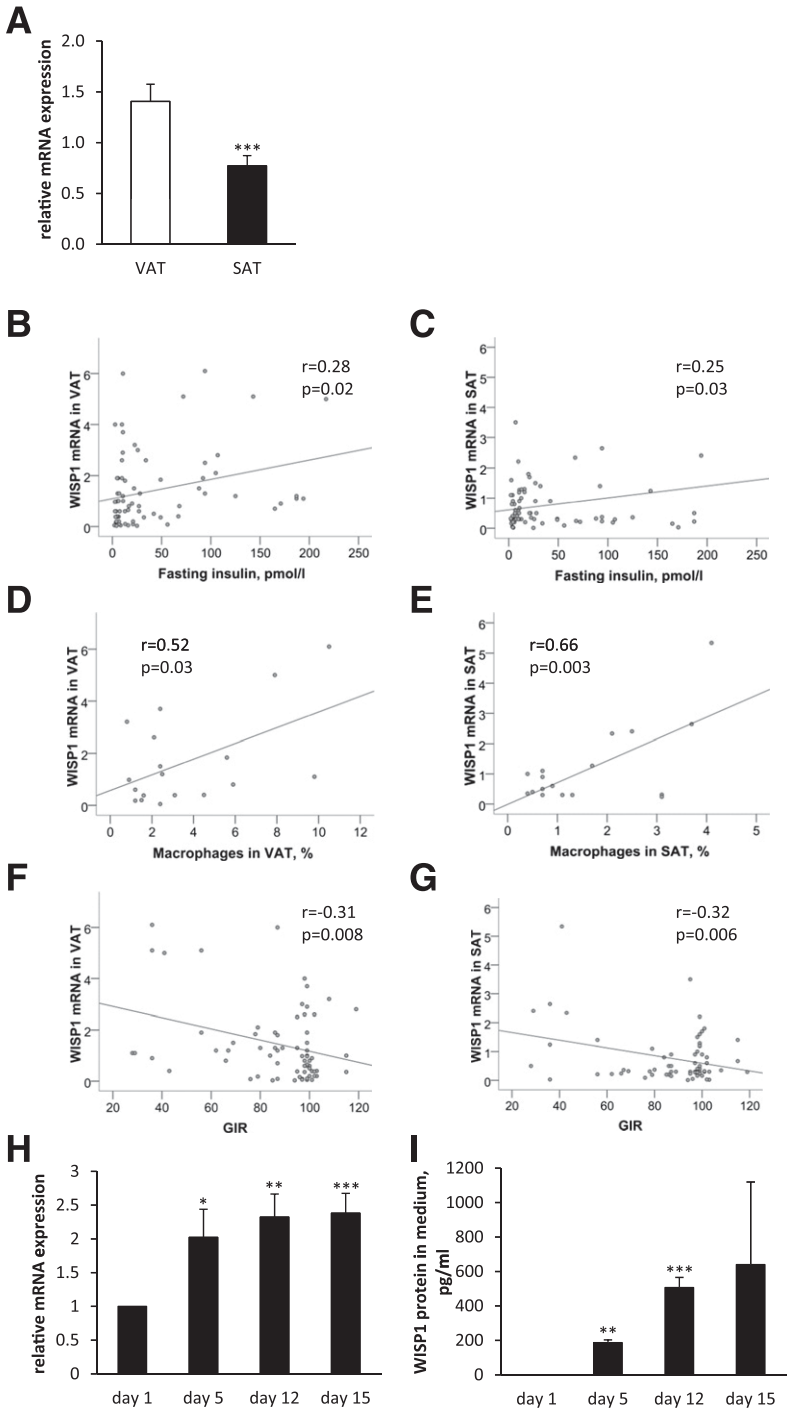

Figure 1—*WISP1* expression in human adipose tissue. In cohort I (n = 75), *WISP1* mRNA expression in VAT and SAT in the normal glucose-tolerant subjects was measured (*A*), and correlations of WISP1 mRNA levels in VAT and SAT with fasting plasma insulin (*B* and *C*), macrophage infiltration in VAT and SAT (*D* and *E*), and insulin sensitivity measured as GIR in the euglycemic clamp (*F* and *G*) were analyzed. In human mesenchymal stem cell–derived adipocytes, *WISP1* mRNA expression (n = 10) (*H*) and WISP1 protein release (n = 3) (*I*) in the culture medium were measured during cell differentiation. Medium samples were concentrated as described in RESEARCH DESIGN AND METHODS. *P < 0.05; **P < 0.01; ***P < 0.001. GIR, glucose infusion rate.

with plasma WISP1 levels (r = 0.73, P = 0.001), and weight loss-induced changes of *WISP1* expression indicated a tendency to correlate with changes of circulating WISP1 (r = 0.49, P = 0.055). Of note, *WISP1* mRNA was significantly decreased after weight loss in female (24.7%, P = 0.02) but not in male (P = 0.83) subjects. Circulating WISP1 also demonstrated a tendency to decrease only in female subjects (27.5%, P = 0.13). Female subjects had more body fat before the diet intervention (P = 0.023) and achieved less reduction of body fat content than male subjects

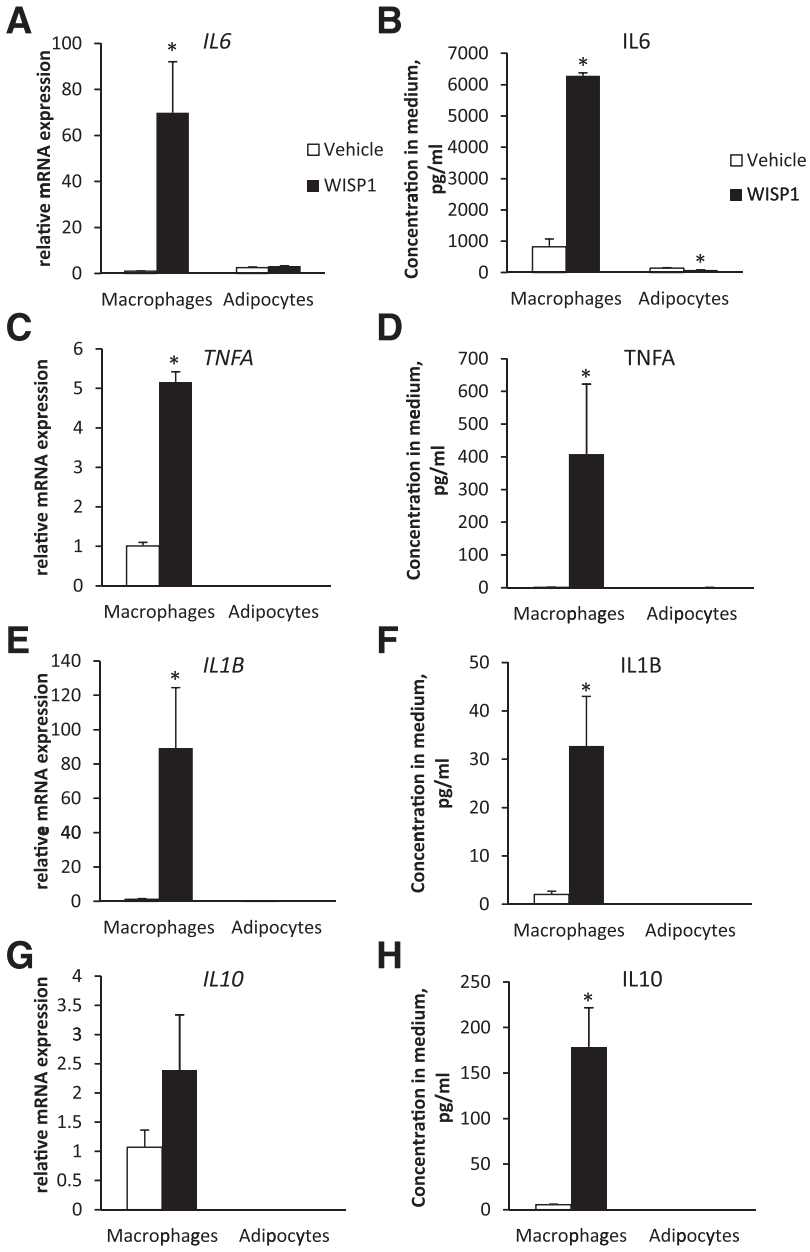

Figure 2—Cytokine secretion in WISP1-treated human adipocytes and macrophages. Human mesenchymal stem cell–derived adipocytes and monocyte-derived macrophages were treated with 0.5 μg/mL WISP1 for 24 h (n = 3). Cytokine mRNA expression measured by qRT-PCR (left) and secretion in culture medium measured by Luminex technology (right) is shown for IL6 (*A* and *B*), TNFA (*C* and *D*), IL1B (*E* and *F*), and IL10 (*G* and *H*). *$P < 0.05$.

(P = 0.001). Of note, women also showed higher WISP1 plasma levels before weight loss (934 ± 199 vs. 2,104 ± 472 pg/mL for male and female subjects, respectively, P = 0.035). No significant differences of other metabolic parameters between males and females before weight loss or in weight loss–induced changes were found.

To test the hypothesis that WISP1 expression is increased in diet-induced obesity, we studied male mice randomized to either a control diet or an HFD. After 6 weeks of diet intervention the HFD-fed mice demonstrated, as expected, increased body weight, fat mass, and lean mass compared with their control counterparts (Fig. 4*C*). *WISP1* expression was upregulated in the epididymal fat tissue, liver, and muscle of HFD-fed mice (Fig. 4*D*).

Hepatic WISP1 Expression Has No Association With Ectopic Fat Accumulation in Obesity

To determine whether ectopic fat accumulation may influence tissue *WISP1* expression in humans, we studied paired samples of liver tissue, SAT, and VAT from non-NASH subjects and patients with NAFLD, which is strongly associated with visceral obesity. Liver and VAT samples both demonstrated higher *WISP1* expression

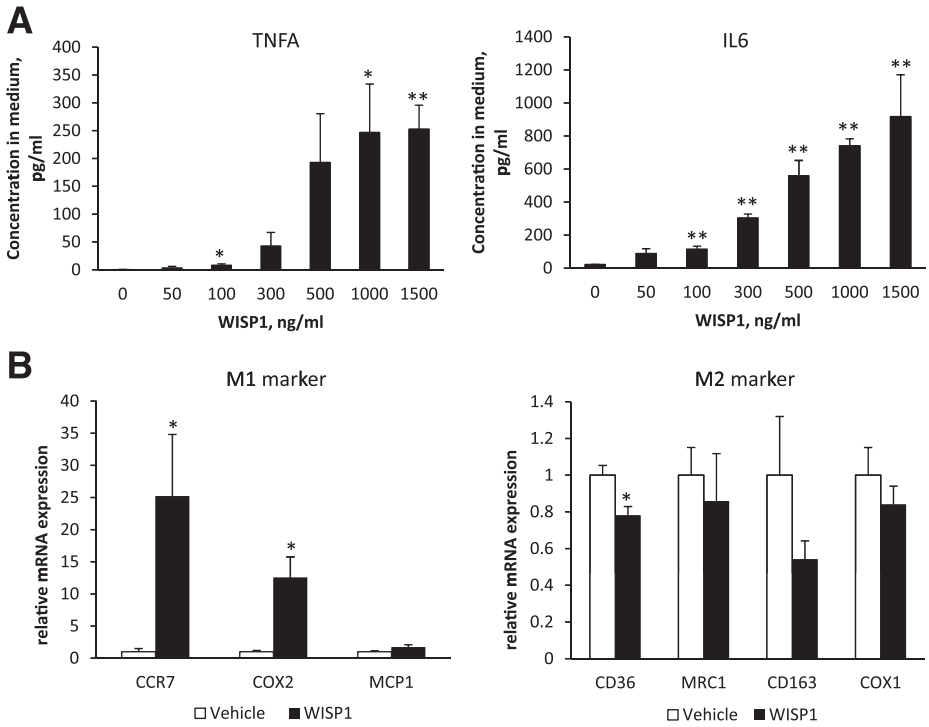

Figure 3—WISP1 effects in macrophages. *A*: Dose-dependent response of cytokine secretion by macrophages to WISP1 treatment. Monocyte-derived macrophages were stimulated with recombinant human WISP1 from 50 to 1,500 ng/mL for 24 h (n = 3). Cytokine secretion in culture medium was measured by Luminex technology. *B*: WISP1 effects on the expression of macrophage polarization markers. Isolated blood monocytes and mature monocyte-derived macrophages were treated with 500 ng/mL WISP1 for 24 h. Gene expression of inflammatory M1 markers *CCR7*, *COX2*, and *MCP1* and anti-inflammatory M2 markers *CD36, MRC1, CD163*, and *COX1* was measured by qRT-PCR. *P < 0.05, **P < 0.01 vs. vehicle.

compared with SAT, and no difference between VAT and liver was found (Fig. 5*A*). Hepatic *WISP1* mRNA was detectable in 65% of non-NASH and 46% of NASH subjects, with no marked sex difference (P = 0.88; data not shown). The groups were also comparable in terms of age and markers of liver or kidney function but significantly differed with respect to BMI, NAS, and fasting insulin levels and varied in trend for HOMA insulin resistance (HOMA-IR) ($P < 0.001$, $P < 0.001$, $P = 0.047$, and $P = 0.060$, respectively) (Table 1). We found neither an association of hepatic *WISP1* expression with BMI ($r = -0.003$, $P = 0.99$), plasma glucose ($r = 0.11$, $P = 0.58$), HOMA-IR ($r = 0.13$, $P = 0.51$), and triglycerides ($r = -0.04$, $P = 0.85$) nor an association with clinical surrogate parameters of liver function (i.e., aspartate transaminase/alanine transaminase ratio) ($r = -0.07$, $P = 0.73$) in this cohort. Accordingly, when compared with non-NASH controls, subjects suffering from histopathologically proven NASH had a similar expression of hepatic *WISP1* ($P = 0.53$) (Fig. 4*B*), and no correlations between hepatic and SAT or VAT *WISP1* expression were observed ($r = 0.12$ [$P = 0.45$] and $r = -0.27$ [$P = 0.86$] for expression in VAT and SAT, respectively). Moreover, regarding *WISP1* expression in VAT and SAT, we found a correlation tendency of VAT *WISP1* mRNA with systolic blood pressure ($r = 0.275$, $P = 0.078$). No further associations with markers of the metabolic syndrome, that is, BMI ($r = 0.039$, $P = 0.80$), fasting glucose ($r = -0.174$, $P = 0.25$), HOMA-IR ($r = -0.136$, $P = 0.37$), or triglycerides ($r = -0.014$, $P = 0.93$), were apparent.

WISP1 is Upregulated by Insulin in Human Adipocytes In Vitro But Not by the Acute Insulin Infusion In Vivo

Gene expression of *WISP1* was increased after stimulation with 100 nmol/L insulin for 4 h (Fig. 6*A*). To elucidate pathways involved in WISP1 regulation by insulin, 3T3-L1 adipocytes were pretreated with vehicle, PI3K inhibitor LY294002, MAPK inhibitor PD098059, or IGF-1R kinase inhibitor NVP-AEW541 30 min before insulin stimulation. All these inhibitors abolished the induction of *WISP1* expression upon insulin treatment (Fig. 6*B*).

In the steady state of the hyperinsulinemic-hyperglycemic clamp experiments, circulating insulin levels increased to 754.4 ± 321.7 pmol/L and were higher than those in the euglycemic-hyperinsulinemic clamp experiments (392.7 ± 77.3 pmol/L, $P < 0.001$). We observed no effect of insulin on *WISP1* expression in SAT in the euglycemic-hyperinsulinemic or hyperinsulinemic-hyperglycemic clamp experiments in overweight, glucose-tolerant subjects in vivo (Fig. 6*C* and *D*).

DISCUSSION

The results show that the CCN family member WISP1 is a novel adipokine released by fully differentiated human adipocytes and stimulated cytokine responses in macrophages.

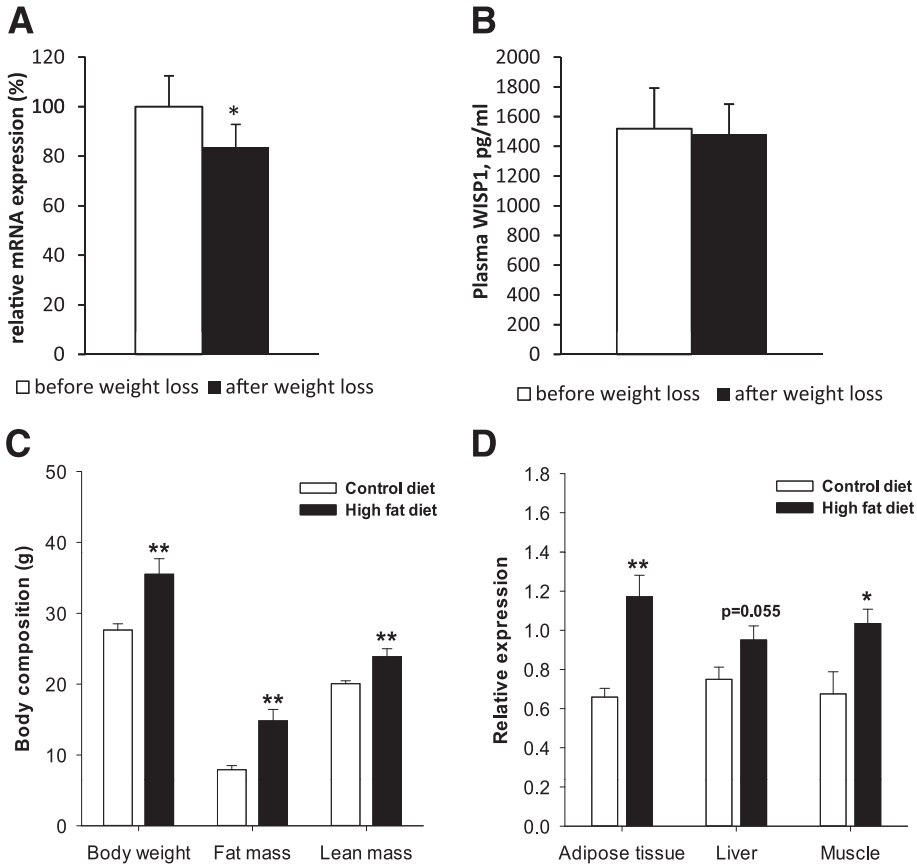

Figure 4—Regulation of *WISP1* expression by body weight changes. *WISP1* mRNA expression in SAT (*A*) and WISP1 protein level in plasma (*B*) of subjects after weight loss in cohort II ($n = 46$). Changes of body weight, body composition (*C*), and *WISP1* mRNA expression in epididymal fat tissue, liver, and muscle (*D*) in mice after 6 weeks of the control diet or HFD ($n = 7$). *$P < 0.05$; **$P < 0.01$.

WISP1 release increases substantially during fat cell differentiation. A comparison of mRNA expression in mature adipocytes with preadipocytes and monocyte-derived macrophages as well as a comparison of adipose and liver tissue showed that adipocytes are likely to be a major source of WISP1 released into the circulation.

WISP1 is involved in the regulation of apoptosis and autophagy in a broad spectrum of neuronal, musculoskeletal, immunologic, and cancer diseases (14). Studies have shown that other CCN family members are closely linked to adipogenesis (5,6,11), but no data exist about the role of WISP1/CCN4 in obesity and associated diseases. We found that *WISP1* gene expression and WISP1 protein production is upregulated during human adipocyte differentiation. Furthermore, we conducted a systematic investigation of *WISP1* gene expression in human paired SAT and VAT samples from a cohort of healthy glucose-tolerant subjects with varying degrees of body weight.

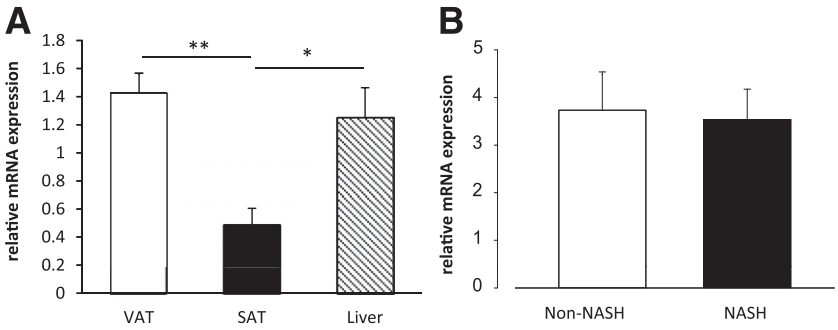

Figure 5—*WISP1* expression in subjects with NAFLD. *WISP1* mRNA expression in paired samples of human liver tissue, SAT, and VAT (*A*) as well as in liver tissue of non-NASH and NASH subjects (*B*) in cohort III ($n = 29$). *$P < 0.05$; **$P < 0.01$.

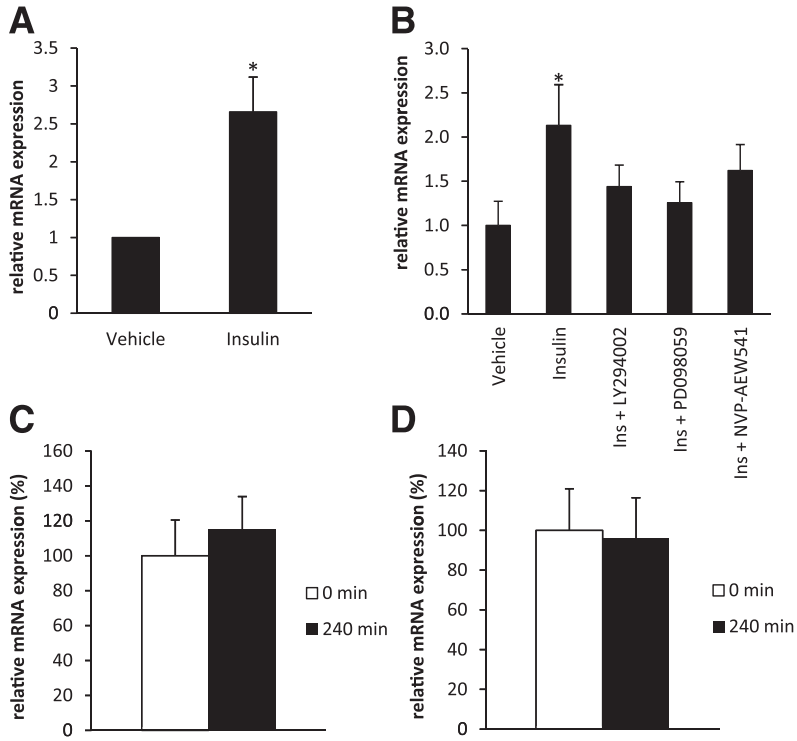

Figure 6—*WISP1* regulation by insulin in adipocytes. *WISP1* mRNA expression in human mesenchymal stem cell–derived adipocytes treated with 100 nmol/L insulin for 4 h (n = 3) (*A*) and in 3T3-L1 adipocytes pretreated with vehicle, PI3K inhibitor LY294002, MAPK inhibitor PD098059, or IGF-1R kinase inhibitor NVP-AEW541 30 min before stimulation with 100 nmol/L insulin for 4 h (n = 3) (*B*). WISP1 mRNA expression in SAT of nondiabetic subjects from cohort IV before and after 4 h of the euglycemic-hyperinsulinemic clamp (n = 10) (*C*) or hyperinsulinemic-hyperglycemic clamp (n = 8) (*D*). *$P < 0.05$ vs. vehicle. Ins, insulin.

In contrast to another CCN family member, WISP2 (12), *WISP1* was highly expressed in VAT and moderately expressed in SAT. Of note, *WISP1* expression correlated negatively with insulin sensitivity, circulating adiponectin levels, and visceral fat content as measured by MRI, suggesting that WISP1 may be a useful marker of visceral fat accumulation and insulin resistance. In support of this hypothesis, we observed only borderline association of *WISP1* expression in SAT and VAT with BMI and waist-to-hip ratio. Moreover, we observed a positive correlation of WISP1 with macrophage infiltration in both SAT and VAT. On the basis of a possible contribution of macrophages to the effects described for adipose tissue, we were unable to detect *WISP1* expression in cultures of primary human monocytes and macrophages.

Cultured macrophages stimulated with WISP1 notably displayed a dose-dependent increase in proinflammatory cytokine production, whereas this was not the case in adipocytes. Moreover, WISP1 modulated macrophage polarization toward the inflammatory M1 phenotype, as was evident from the induction of the expression of M1 markers CCR7 and COX2, whereas expression of anti-inflammatory M2 markers was decreased. Furthermore, WISP1 caused a dose-dependent increase in extracellular matrix–degrading enzyme mRNA expression, such as that measured for ADAMTS-4 and matrix metalloproteinases MMP-3, MMP-9, and MMP-13 in murine RAW 264.7 macrophages (26). Thus, adipocyte-derived WISP1 might in this way participate in the control of macrophage function and migration in fat tissue. Because WISP1 is associated with insulin sensitivity, it may be characterized as an adipokine that participates in the control of macrophage function.

We observed reduced *WISP1* gene expression in SAT and decreased circulating WISP1 levels after weight reduction in female subjects, who had more body fat and higher WISP1 plasma levels before weight loss than male subjects. Although human white adipose tissue displays a high adipocyte turnover, the total number of adipocytes in adults remains constant even after weight reduction (27). Thus, the observed downregulation in SAT likely reflects a decreased expression of *WISP1* in adipocytes but not a decreased adipocyte number. Additionally, *WISP1* expression in liver was moderate and not upregulated in the subjects with NAFLD in the present study, and we observed no association between various biochemical and anthropometrical markers of obesity and hepatic gene expression of *WISP*, suggesting that WISP1 is not involved in hepatic fat accumulation. Thus, the weight loss–induced changes in circulating WISP1 most likely originated from adipose tissue rather than from the liver or other organs. Another CCN family member, NOV, also is associated with obesity, and NOV levels in circulation are decreased after weight reduction due to bariatric

surgery (11). In line with the present data, circulating NOV levels are higher in women than in men (11). Thus, the sex differences in the changes of WISP1 concentration after weight loss in the present study might reflect regulation of CCN peptide production by sex hormones (28) and needs further investigation. The reduction in circulating CCN proteins after weight loss could reflect downregulation of the WNT signaling pathway in various tissues, such as adipose tissue and muscle. WISP1 increase has been found in a variety of cancers (17), and the novel variant of WISP1 is associated with invasion of cholangiocarcinomas (18). Central obesity and the metabolic syndrome are independent risk factors for cancer (29). The effects of circulating WISP1 on tissue regeneration and proliferation are not studied in vivo, but given the proliferative properties of WISP1, they may be a marker of malignancy risk in obesity, which deserves further investigation.

Insulin is an important regulator of adipocyte differentiation and function (30), and the cross talk between insulin and the WNT signaling pathway occurs at multiple levels in murine preadipocytes (31). Antiapoptotic and proliferative effects of WISP1 are mediated through the PI3K/Akt pathway (14), suggesting that WISP1 may affect insulin signaling. We did not, however, observe such an impact of WISP1 in vitro, whereas we did find that insulin signaling increases *WISP1* gene expression in human adipocytes. This finding could not be easily translated to the in vivo situation because short exposition of patients/subjects to hyperinsulinemia during clamp experiments had no effect on *WISP1* expression in SAT. Several causes may explain the observed discrepancy between the in vitro and in vivo experiments. First, the systemic insulin concentration during clamp experiments does not reflect the local intratissue insulin concentration, which was possibly lower compared with the insulin concentration in cell experiments. Second, insulin induces multiple metabolic changes in the body, which can influence *WISP1* expression and/or production and minimize the direct effect of insulin on adipocytes. Third, the subjects investigated were moderately insulin resistant and, thus, permanently exposed to elevated insulin levels during the clamps, whereas cell cultures were insulin deprived and then exposed to much higher doses of insulin. Because the cell culture data demonstrate the ability of insulin to augment *WISP1* expression, in vivo effects are probably affected by chronic exposure and are slower and depend less on dosage.

Taken together, the present data show that WISP1 is a novel adipokine that is substantially overexpressed in visceral fat from obese subjects and reflects insulin resistance and adipose tissue inflammation. Weight changes regulate circulating WISP1 levels and WISP1 expression in adipose tissue. Therefore, we propose that WISP1 is a novel link between obesity and inflammation.

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