WISP1 is a novel adipokine linked to inflammation in obesity

Short title: WISP1 and central obesity

Veronica Murahovschi¹,²*, Olga Pivovarova¹,²*, Iryna Ilkavets³, Renata M. Dmitrieva⁴,
Stephanie Döcke¹, Farnaz Keyhani-Nejad¹,², Özlem Gögebakan¹,², Martin Osterhoff¹,²,
Margrit Kemper¹,², Silke Hornemann¹,², Mariya Markova¹,², Nora Klöting⁶, Martin
Stockmann⁷, Martin O. Weickert⁸, Valeria Lamounier-Zepter⁹, Peter Neuhaus⁷, Alexandra
Konradi⁴, Steven Dooley³, Christian von Loeffelholz¹,⁵, Matthias Blüher⁶, Andreas F. H.
Pfeiffer¹,², Natalia Rudovich¹,²

¹Department of Clinical Nutrition, German Institute of Human Nutrition Potsdam-
Rehbruecke, Nuthetal, Germany;

²Department of Endocrinology, Diabetes and Nutrition, Campus Benjamin Franklin, Charité –
Universitätsmedizin, Berlin, Germany;

³Department of Medicine II, Section Molecular Hepatology – Alcohol Associated Diseases,
Medical Faculty Mannheim, University of Heidelberg, Germany;

⁴Almazov Federal Medical Research Centre, Saint-Petersburg, Russian Federation;

⁵Integrated Research and Treatment Center, Center for Sepsis Control and Care (CSCC),
Friedrich Schiller University, and Department of Anesthesiology and Intensive Care, Jena
University Hospital, Jena, Germany;

⁶Department of Medicine, University of Leipzig, Leipzig, Germany;
7Department of General, Visceral and Transplantation Surgery, Charité – Universitätsmedizin, Berlin, Germany;

8Warwickshire Institute for the Study of Diabetes, Endocrinology and Metabolism, University Hospitals Coventry and Warwickshire NHS Trust, Coventry, UK; and Division of Metabolic & Vascular Health, Warwick Medical School, University of Warwick, Coventry, UK;

9Medical Clinic III, Dresden University of Technology, Dresden, Germany.

*V.M. and O.P. contributed equally to this manuscript.

**Corresponding author:** Dr. Natalia Rudovich
German Institute of Human Nutrition Potsdam
Arthur-Scheunert-Street 114-116
14558 Nuthetal, Germany
Tel: +49 33 2008 82771
Fax: +49 33 2008 82777
E-mail address: rudovich@dife.de

**Word count:** abstract - 196, main text - 4000

**Number of figures and tables:** 6 and 1
ABSTRACT

WISP1 (Wnt1 inducible signaling pathway protein 1, CCN4) is a member of the secreted extracellular matrix-associated proteins of the CCN family and 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. Here we aim to validate WISP1 as a novel adipokine.

In our study, human adipocyte differentiation was associated with increased WISP1 expression and secretion. Stimulation of human macrophages with WISP1 led to pro-inflammatory response. Circulating WISP1 and WISP1 subcutaneous adipose tissue expression were regulated by weight changes in humans and mice. WISP1 expression in visceral and in 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 between 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. Our data suggests 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 and 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 “metainflammation” 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 are acting in both autocrine and paracrine fashions to regulate cell proliferation, cell fate, differentiation and organism development (8, 9). The WNT signaling network consists of multiple so-called “canonical” and “non-canonical” pathways which 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 of the canonical WNT signaling pathway (10). Experimental evidence suggests that the 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 which bear analogy to various extracellular protein domains. Module I is homologous to the insulin-like growth factor (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 C-terminal domain (13). WISP1 is expressed in various organs and tissues including heart, pancreas, lung, kidney, small intestine, ovaries, spleen and brain and, in some of these tissues,
acts anti-apoptotic through PI3K and Akt pathways (14). WISP1 possesses 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 effects of WISP1 on insulin target tissues, including liver and fat. In the present study, we combined in vitro experiments with four independent clinical studies, aiming 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 was substantially elevated in visceral fat tissue (VAT) rather than in subcutaneous fat tissue (SAT) in glucose tolerant subjects; 3) WISPI expression correlated with insulin sensitivity, adiponectin and markers of adipose tissue inflammation; 4) weight reduction decreased WISPI expression in SAT as well as circulating WISP1 levels in plasma; and 5) hepatic WISPI expression showed 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 (26). Percentage body fat was measured by dual-energy X-ray absorptiometry (DEXA). In the subgroup \((n=52)\), abdominal visceral fat content was measured by MRI as described previously (27). Insulin sensitivity was assessed with the euglycemic-hyperinsulinemic clamp method as described previously (28). Macrophage content in VAT and SAT samples was visualized in adipose tissue sections (stained with hematoxylin and eosin) by additional staining against CD68 \((1:200; \text{DAKO})\) as described previously (26). A total of 100 cells were studied from each slide and CD68 positive cells were counted. Adipocyte cell size in VAT and SAT were analysed in adipose tissue sections as described previously (26).

Cohort II

Effects of weight reduction were studied in 49 subjects kept on an 8-week low-calorie diet (Modifast, Nutrition et Santé, France) consisting of 800 kcal/d plus 200 g/d 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 (19). Percentage of body fat was measured by dual-energy X-ray absorptiometry. Plasma samples and subcutaneous adipose tissue biopsies were collected after overnight fasting before and after weight loss.

Cohort III

In our human liver study, 47 patients met the inclusion criteria, gave informed consent and were enrolled as recently detailed (29). Comparable to liver tissue, perfused samples of VAT
and abdominal SAT were harvested by knife extraction before therapeutical interventions. All samples were flash-frozen in liquid nitrogen and stored at -80°C until 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 non-alcoholic fatty liver disease (NAFLD) score and to identify subjects with undefined and definite non-alcoholic steatohepatitis (NASH) (29). Study subjects were accordingly considered to suffer from undefined or definite NASH (NAS≥3), or as non-NASH subjects (NAS<3). Exclusively healthy liver tissue was used for analyses, as a blinded clinical expert pathologist considered all harvested liver samples to be histologically normal with respect to pathologies except for 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: (i) control experiments (0.9% saline infusion (SC), n=8); (ii) hyperinsulinemic-euglycemic clamp (EC) for 4 h with continuous infusion of 40·mU·m² of the body surface·min⁻¹ human insulin at a steady state capillary plasma glucose concentration of 4.4 mmol/l (80 mg/dl) (n=10); (iii) hyperinsulinemic-hyperglycemic clamp (HC) for 4 h with continuous infusion of 40·mU·m² of the 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 (30). 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 subcutaneous adipose tissue biopsies were taken before and after infusions (t=-40 min and +240 min).
In cohorts I and III, paired samples of visceral adipose tissue and subcutaneous adipose tissue were obtained from the same sites during abdominal surgery by knife extraction. In cohorts II and IV, subcutaneous adipose tissue biopsies 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, GA, USA). For this, medium samples were concentrated using Vivaspin 2 concentrators (Sartorius, Germany). Cytokine release in cell culture medium was quantified using ProcartaPlexTM Multiplex Immunoassays (eBioscience, Germany).

**Animal study**

Animal protocols were approved by the local governmental animal ethic 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 (60 kcal-% fat, 20 kcal-% protein, 20 kcal-% carbohydrate, 5.24 kcal/g) (Research Diets, Inc., USA) containing 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, USA) and 50 ng/ml of human granulocyte-macrophage colony-stimulating factor (Peprotech, Germany) for 7 days and stimulated with recombinant human WISP1 (E.Coli, Endotoxin < 0,1 ng/µg of protein; Peprotech, Germany) from 50 ng/ml to 1.5 µg/ml for 24h.

Human mesenchymal stem cells (hMSCs) were isolated from subcutaneous abdominal adipose tissue samples (31) and differentiated into mature adipocytes in DMEM-F12 supplemented with 500 µM 3-isobutyl-1-methylxanthine (IBMX), 25 nM dexamethasone, 0.2 nM 3,3,5-triiodo-L-thyronine, 8 µg/ml D-biotin, 15 mM D-pantothenat, 100 nM hydrocortisone, 20 nM insulin, 0.01 mg/ml transferrin (all from Sigma-Aldrich, Germany) and 2 µM rosiglitazone (Cayman Chemical, USA) for 14 days. Adipocyte morphology was confirmed by oil red O staining (Suppl. Figure 1A). Differentiated adipocytes were treated with 100nmol/L insulin (Sigma-Aldrich, Germany) for 4h or with 0.5µg/ml WISP1 (Peprotech, Germany) for 24h.

3T3-L1 adipocytes were differentiated in DMEM supplemented with 10% HyClone FCS (Thermo Scientific, USA), 1µg/L insulin, 0.5mmol/L IBMX and 0.25µmol/L dexamethasone for 7 days and treated with or without LY 294002 (25µM, Sigma-Aldrich, Germany), PD 098059 (30µM, Sigma-Aldrich, Germany) and NVP-AEW541 (0.1µM, Cayman Chemicals) 30 min before the stimulation with 100nmol/L insulin for 4h.

Oil red 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 3 parts of Oil Red O stock solution (Sigma-Aldrich, Germany) with 2 parts DI water. Further 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 washed with tap water until the water rinses off clear.

**Quantitative real-time PCR (qRT-PCR)**

Total RNA was extracted by NucleoSpin® RNA II (Macherey-Nagel, Germany) Kit or RNeasy Lipid Tissue Mini Kit (Qiagen, Hilden, Germany). Synthesis of cDNA was performed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA). QRT-PCR was performed using Power SYBR Green PCR Master Mix (Applied Biosystems, USA) and primers shown in Suppl. Table 1.

**Western blotting**

Semi-dry immunoblots were performed with antibodies specific to phospho-p44/42 MAPK (pERK), p44/42 MAPK (ERK), phospho-Akt and Akt (Life Technologies/Cell Signalling, USA), WISP1 (ab10737, Abcam, Cambridge, MA), α-tubulin (Life Technologies/Cell Signalling, USA) and IRDye® 800CW Goat anti-Rabbit IgM (LI-COR Bioscience, USA) and quantified by Odyssey® Infrared Imaging System (LI-COR, Germany).

**Statistical Analysis**

SPSS 20.0 (Chicago, USA) was used for all statistical analyses. If not stated otherwise, data are given as means±S.E.M. Presence or absence of normal distribution was verified by the Kolmogorov-Smirnov test. Depending on the distribution of data, Pearson’s simple coefficient or Spearman’s rank correlation coefficient was used for correlation analysis, and Mann-Whitney-U test or Student’s t-test was applied to estimate differences between groups. P-value of < 0.05 was considered significant.
RESULTS

WISP1 gene expression in subcutaneous and visceral adipose tissue is associated with markers of insulin sensitivity and adipose tissue inflammation

Baseline characteristics of the cohort’s I-IV are summarised in Table 1. WISP1-mRNA expression in VAT was higher than in SAT in normal glucose tolerant subjects in the cohort I (Figure 1-A). We hypothesized that the level of WISP-1-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 in SAT, respectively), with macrophage infiltration ($r=0.52$, $p=0.03$ and $r=0.66$, $p=0.003$ for expression in VAT and in SAT, respectively) and negatively with insulin sensitivity, measured as Glucose Infusion Rate in the euglycemic - hyperinsulinemic clamp ($r=-0.31$, $p=0.008$ and $r=-0.32$, $p=0.006$, for expression in VAT and SAT respectively) (Figure 1B-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), with waist-to-hip ratio ($r=0.17$, $p=0.15$ and $r=0.20$, $p=0.08$ for VAT and SAT, respectively), with 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 (Figure 1-H and Suppl. Fig. 1-A-B). The increase of WISP1 mRNA
expression during adipocyte differentiation was accompanied by an elevation of intracellular WISP1 protein expression (Suppl. 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 hours. In the macrophage culture, we observed a significant and dose-dependent, WISP1-induced increase of IL6, TNFA, IL1B, and IL10 at mRNA expression level, as well as at protein level in the culture medium (Fig. 2, Fig. 3-A). Moreover, WISP1 induced an increase of the expression of inflammatory M1 markers of macrophage polarisation, CCR7 and COX2. In line with this, expression of M2 marker CD36 and in tendency also CD163 were 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 mRNA level or in the culture medium. It is of note that 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 the oil red staining (Suppl. Figure 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 (Suppl. Fig. 2).

WISP1 is inversely regulated by high fat diet induced obesity and weight loss

We next examined the expression of WISP1 in the SAT of subjects who underwent weight loss using a low-caloric 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-B). Nevertheless, basal WISP1 mRNA expression in
SAT strongly correlated with plasma WISP1 levels ($r=0.73$, $p=0.001$), and weight loss-induced changes of $WISPI$ expression indicated tendency to correlation with changes of circulating WISP1 ($r=0.49$, $p=0.055$). Interestingly, $WISPI$ mRNA was significantly decreased after weight loss only in female (24.7%, $p=0.02$), but not in male subjects ($p=0.83$). Circulating WISP1 also demonstrated a tendency to decrease only in female subjects (27.5%, $p=0.13$). Female subjects had more body fat before diet intervention ($p=0.023$) and archived less reduction of body fat content than male subjects ($p=0.001$). Interestingly, women also showed higher WISP1 plasma level before weight loss (934±199 pg/ml vs. 2104±472 pg/ml, $p=0.035$, for male and female subjects, respectively). 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, which were randomized into either a control diet or high fat diet (HFD). After 6 weeks of diet intervention, as expected, the HFD-fed mice demonstrated increased body weight, fat mass and lean mass compared with their control counterparts (Figure 4-C). $WISPI$ expression was up-regulated in the epididymal fat tissue, liver and muscle of HFD-fed mice (Figure 4-D).

**Hepatic WISP1 expression has no association with ectopic fat accumulation in obesity**

To determine whether ectopic fat accumulation may influence tissue $WISPI$ expression in humans, we studied paired samples of liver, SAT and VAT tissue from non-NASH subjects and patients with nonalcoholic fatty liver disease (NAFLD), where the disease is strongly associated with visceral obesity. Liver samples and VAT both demonstrated higher $WISPI$ expression when compared with SAT, and no difference with VAT (Figure 5-A). Hepatic $WISPI$ mRNA was detectable in 65% of non-NASH and 46% of NASH subjects, with no
marked gender 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, NAFLD activity score, fasting insulin levels and varied in trend for 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 ($r=0.13$; $p=0.51$), triglycerides ($r=-0.04$; $p=0.85$), nor with clinical surrogate parameters of liver function, i.e. AST/ALT ratio ($r=-0.07$; $p=0.73$) in this cohort. Accordingly, when compared to non-NASH controls, subjects suffering from histopathologically proven NASH had a similar expression of hepatic $WISP1$ ($p=0.53$; Figure 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 an indication of a tendency to correlation of VAT $WISP1$ mRNA with systolic blood pressure ($r=0.275$, $p=0.078$). No further associations with markers of the metabolic syndrome became apparent, i.e. 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 ($r=-0.014$; $p=0.93$).

*WISP1 is up-regulated 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 100nM insulin for 4 hours (Figure 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 (Figure 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 in the hyperinsulinemic-euglycemic clamp (392.7±77.3 pmol/l; p<0.001). We observed no effect of insulin on \textit{WISP1} expression in SAT in the hyperinsulinemic-euglycemic or hyperinsulinemic-hyperglycemic clamp in overweight glucose-tolerant subjects \textit{in vivo} (Figure 6 C-D).
DISCUSSION

Our results show that the CCN family member WISP1 is a novel adipokine which is released by fully differentiated human adipocytes and stimulated cytokine responses in macrophages. WISP1 release increases substantially during fat cell differentiation. A comparison of mRNA expression in mature adipocytes with pre-adipocytes and monocyte-derived macrophages as well as the 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). Recent studies show 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 WISPI gene expression and WISP1 protein production is up-regulated during human adipocyte differentiation. Further, we conducted a systematic investigation of WISPI gene expression in human paired SAT and VAT samples, in a cohort of healthy glucose-tolerant subjects with different degrees of body weight. In contrast to another CCN family member, WISP2 (12), WISPI was highly expressed in VAT and moderately expressed in SAT. Interestingly, WISPI expression correlated negatively with insulin sensitivity, circulating adiponectin levels and with 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 WISPI 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. Based on the possible contribution of macrophages to effects described in adipose tissue, we also examined WISPI expression in cultures of primary human monocytes and macrophages and were unable to detect WISPI.
Interestingly, cultured macrophages stimulated with WISP1 displayed a dose-dependent increase in pro-inflammatory cytokine production, whereas this was not the case in adipocytes. Moreover, WISP1 modulated macrophage polarization towards inflammatory M1 phenotype as was evident from induction of the expression of M1 markers CCR7 and COX2, whereas expression of anti-inflammatory M2 markers was decreased. Further, WISP-1 caused a dose-dependent increase in extracellular matrix degrading enzyme mRNA expression such as measured for ADAMTS-4, MMP-3, MMP-9, and MMP-13 in murine RAW 264.7 macrophages (20). Thus, adipocyte-derived WISP1 might in this way participate in the control of macrophage function and migration in fat tissue. Since 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 participants. Interestingly, female subjects had more body fat and higher WISP1 plasma level before weight loss in comparison with male subjects. While human white adipose tissue displays a high adipocyte turnover, the total number of adipocytes in adults remains constant even after weight reduction (21). Thus, it is likely that the observed down-regulation in SAT reflected decreased expression of WISP1 in adipocytes but not a decreased adipocyte number. Additionally, WISP1 expression in liver was moderate and was not up-regulated in the subjects with NAFLD in our study. Furthermore, we observed no association between different 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 originated most likely from adipose tissue rather than from the liver or other organs. Interestingly, another CCN family member, NOV, is associated with obesity and NOV levels in circulation are decreased after weight reduction due to bariatric surgery (11). In line with
our data, circulating NOV levels are higher in women when compared with male subjects (11). Thus, the gender differences in the changes of WISP1 concentration after weight loss in our study might reflect regulation of CCN peptides production by sexual hormones (22) and needs further investigation. The reduction in circulating CCN proteins after weight loss could reflect down-regulation of the WNT-signalling pathway in different tissues such as adipose tissue and muscle in these subjects. 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 metabolic syndrome are independent risk factors for cancer (23). The effects of circulating WISP1 on tissue regeneration and proliferation are not studied in vivo, but given the proliferative properties of WISP1, it may be a marker of malignancy risk in obesity, which deserves further investigation.

Insulin is an important regulator of adipocyte differentiation and function (24) and the cross-talk between insulin and the WNT signalling pathway occurs at multiple levels in murine preadipocytes (25). Anti-apoptotic and proliferative effects of WISP1 are mediated via the PI3K/Akt pathway (14), suggesting that WISP1 may affect insulin signaling. We did, however, not observe such impact of WISP1 in vitro, whereas we found that the other way around, insulin signalling increases WISP1 gene expression in human adipocytes. This finding could not be easily translated to the in vivo situation, since 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 in vitro and in vivo experiments: firstly, the systemic insulin concentration during clamp experiments does not reflect the local intra-tissue insulin concentration, which was possibly lower compared to the insulin concentration in cell experiments; secondly, 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. Moreover, the subjects investigated were moderately
insulin-resistant and thus permanently exposed to elevated insulin levels during the clamps while cell cultures were insulin-deprived and then exposed to much higher doses of insulin. Since the cell culture data prove the ability of insulin to augment \textit{WISP1} expression, \textit{in vivo} effects are probably affected by chronic exposure, are slower and less dependent on dosage.

Taken together, our data shows 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.
Acknowledgments

We thank Ms. June Inderthal from Department of Clinical Nutrition, German Institute of Human Nutrition Potsdam for reading the manuscript. The authors would like to thank PD Dr. Arne Dietrich from Department of Surgery, University of Leipzig for providing adipose tissue samples, and Dr. Anna Kipp and Dr. Tim J. Schulz from German Institute of Human Nutrition Potsdam for critical discussion of the manuscript.

This work was supported by the Kompetenznetz Adipositas (Competence network for Obesity) funded by the Federal Ministry of Education and Research (German Obesity Biomaterial Bank; FKZ 01GI1128 for M. B. and FKZ 01GI1122G for V. L. Z.), The Virtual Liver Network (S. D.), GERUSSFIT (V. M., R. D., A. K., A. F. H. P., N. R.), Metabolic Genome Profile (MGP 0313042C/ N. R., A. F. H. P.), grants from Deutsche Forschungsgemeinschaft (DFG), the SFB 1052/1: “Obesity mechanisms” (project B01) (M. B.) and DFG grant No.Pf164/021002 (N. R., Ö. G., A. F. H. P.), and by the Helmholtz Alliance ICEMED – Imaging and Curing Environmental Metabolic Diseases, through the Initiative and Networking Fund of the Helmholtz Association (M. B).

No potential conflicts of interest relevant to this manuscript were reported.

V. M., O. P., A. F. H. P. and N. R. wrote the manuscript and researched data. I. I., R. M. D., S. D., F. K-N, M. M., Ö. G., M. O., N. K., M. S., M. O. W., V. L.-Z., P. N., S. D., A. K., C. v L., M. B. researched data and reviewed and edited the manuscript. A. F. H. P. and N. R. are guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
References


Figure 1. WISP1 expression in human adipose tissue.

In the 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, C), macrophage infiltration in VAT and SAT (D, E), and insulin sensitivity (F, G) were analysed. In human mesenchymal stem cell derived adipocytes, WISP1 mRNA expression (H, n = 10) and WISP1 protein release in the culture medium (I, n = 3) 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.

Figure 2. Cytokine secretion in WISP1-treated human adipocytes and macrophages

Human mesenchymal stem cell derived adipocytes and monocyte-derived macrophages were treated with 0.5µg/ml WISP1 for 24h (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, B), TNFA (C, D), IL1B (E, F), and IL10 (G, H). * p<0.05.

Figure 3. WISP1 effects in macrophages

(A) Dose-dependence response of cytokine secretion by macrophages to WISP1 treatment. Monocyte-derived macrophages (MDM) were stimulated with recombinant human WISP1 from 50 ng/ml to 1500 ng/ml for 24h (n = 3). Cytokine secretion in culture medium measured by Luminex technology. (B) WISP1 effects on the expression of macrophage polarisation markers. Isolated blood monocytes and mature MDM were treated with 500ng/ml WISP1 for 24h. Gene expression of inflammatory M1 markers, CCR7, COX2, MCP1, and anti-
inflammatory M2 markers, CD36, MRC1, CD163 and COX1 was measured by qRT-PCR; * p<0.05, ** p<0.01 vs. vehicle.

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 the 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 high fat diet (n = 7). *p < 0.05, **p < 0.01.

Figure 5. WISP1 expression in subjects with NAFLD

WISP1 mRNA expression in paired samples of human liver, SAT and VAT tissue (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 100nM insulin for 4h (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 the stimulation with 100nM insulin for 4h (n = 3, B). WISP1 mRNA expression in SAT of non-diabetic subjects from the cohort IV before and after 4h of the hyperinsulinemic-euglycemic clamp (n = 10, C) or hyperinsulinemic-hyperglycemic clamp (n = 8, D). * p < 0.05 vs. vehicle.
Supplemental Figure 1. Differentiation of human mesenchymal stem cell derived adipocytes.

(A) Expression of adipocyte markers PPARG and GLUT4 at different stages of differentiation of human mesenchymal stem cell (hMSC) derived adipocytes measured by real-time PCR (n = 3). (B) Oil Red O staining following adipocyte differentiation in the presence of vehicle (top) or 100ng/ml WISP1 (bottom). Representative phase-contrast photographs are shown. Differentiation of hMSC into adipocytes evidenced by the lipid accumulation in the cytosol of the cells (at least 80% of cells in each well). (C) Expression of WISP1 protein at different stages of adipocyte differentiation in the presence of vehicle or 100ng/ml WISP1. Representative western blots are shown. * p < 0.05, ** p < 0.01.

Supplemental Figure 2. WISP1 effects on the insulin signaling

Western blots (A) and densitometric analysis of total and phosphorylated Akt protein levels (B) or total and phosphorylated ERK (44 and 42 kDa) protein levels (C) in extracts from 3T3-L1 adipocytes treated with 100 nM insulin for 30 min with or without pretreatment with 500ng/ml recombinant WISP1 protein for 30 min (n = 3). * p < 0.05, ** p < 0.01.

Supplemental Table 1. Primers used for real-time PCR
Table 1. Clinical characteristics of studied cohorts

<table>
<thead>
<tr>
<th></th>
<th>Cohort I</th>
<th>Cohort II</th>
<th>Cohort III</th>
<th>Cohort IV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-NASH (NAS &lt; 3)</td>
<td>NASH (NAS ≥ 3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N (% male)</td>
<td>75 (53)</td>
<td>49 (38)</td>
<td>34 (41)</td>
<td>13 (38)</td>
</tr>
<tr>
<td>T2D (n)</td>
<td>0</td>
<td>1</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Age [years]</td>
<td>51.9 ± 1.6</td>
<td>40.4 ± 0.8</td>
<td>58±3</td>
<td>62±3</td>
</tr>
<tr>
<td>BMI [kg/m²]</td>
<td>24.0 ± 0.1</td>
<td>34.5 ± 0.6</td>
<td>24.2±0.6</td>
<td>32.1±2.1**</td>
</tr>
<tr>
<td>Body fat [%]</td>
<td>20.8 ± 0.3</td>
<td>38.6 ± 1.4</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Fasting plasma glucose [mmol/l]</td>
<td>5.3 ± 0.1</td>
<td>5.2 ± 0.1</td>
<td>5.5±0.3</td>
<td>6.4±1.1</td>
</tr>
<tr>
<td>Fasting plasma insulin [pmol/l]</td>
<td>41.0 ± 6.3</td>
<td>78.5 ± 20.1</td>
<td>50.3 ± 7.3</td>
<td>84.6 ± 16.8*</td>
</tr>
<tr>
<td>HOMA_{IR} [mmol · mU · l⁻²]</td>
<td>1.59 ± 0.24</td>
<td>3.82 ± 1.06</td>
<td>2.08±0.40</td>
<td>4.61±1.34</td>
</tr>
<tr>
<td>AST/ALT ratio</td>
<td>ND</td>
<td>ND</td>
<td>1.44±0.17</td>
<td>1.42±0.33</td>
</tr>
<tr>
<td>NAS (0-8)</td>
<td>ND</td>
<td>ND</td>
<td>0.94±0.14</td>
<td>3.88±0.38**</td>
</tr>
</tbody>
</table>
Means ± S.E.M., percentage or absolute numbers of subjects are shown. Statistical differences were determined by Mann-Whitney U test. * p < 0.05 vs. control subjects in cohort III; **p < 0.01 vs. control subjects in cohort III. a– only data before weight loss intervention are shown. ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; NAS, non-alcoholic fatty liver disease (NAFLD) score; T2D, type 2 diabetes mellitus. ND – not done.
**Diabetes**

### A

![Bar chart comparing relative mRNA expression in VAT and SAT](image)

### B

![Scatter plot showing WISP1 mRNA expression in VAT vs. fasting insulin](image)

- $r = 0.28$
- $p = 0.03$

### C

![Scatter plot showing WISP1 mRNA expression in SAT vs. fasting insulin](image)

- $r = 0.25$
- $p = 0.03$

### D

![Scatter plot showing WISP1 mRNA expression in VAT vs. macrophages in VAT](image)

- $r = 0.52$
- $p = 0.03$

### E

![Scatter plot showing WISP1 mRNA expression in SAT vs. macrophages in SAT](image)

- $r = 0.66$
- $p = 0.003$

### F

![Scatter plot showing WISP1 mRNA expression in VAT vs. GIR](image)

- $r = -0.31$
- $p = 0.008$

### G

![Scatter plot showing WISP1 mRNA expression in SAT vs. GIR](image)

- $r = -0.32$
- $p = 0.006$

### H

![Relative mRNA expression over days](image)

- Day 1: *
- Day 5: **
- Day 12: ***
- Day 15: ***

### I

![WISP1 protein in medium over days](image)

- Day 1: *
- Day 5: **
- Day 12: ***
- Day 15: ***
Figure 1. *WISP1* expression in human adipose tissue.
In the 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, C), macrophage infiltration in VAT and SAT (D, E), and insulin sensitivity (F, G) were analysed. In human mesenchymal stem cell derived adipocytes, WISP1 mRNA expression (H, n=10) and WISP1 protein release in the culture medium (I, n=3) 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.
Figure 2. Cytokine secretion in WISP1-treated human adipocytes and macrophages

Human mesenchymal stem cell derived adipocytes and monocyte-derived macrophages were treated with 0.5µg/ml WISP1 for 24h (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, B), TNFA (C, D), IL1B (E, F), and IL10 (G, H). * p<0.05.
Figure 3. WISP1 effects in macrophages

(A) Dose-dependence response of cytokine secretion by macrophages to WISP1 treatment. Monocyte-derived macrophages (MDM) were stimulated with recombinant human WISP1 from 50 ng/ml to 1500 ng/ml for 24h (n = 3). Cytokine secretion in culture medium measured by Luminex technology. (B) WISP1 effects on the expression of macrophage polarisation markers. Isolated blood monocytes and mature MDM were treated with 500ng/ml WISP1 for 24h. Gene expression of inflammatory M1 markers, CCR7, COX2, MCP1, and anti-inflammatory M2 markers, CD36, MRC1, CD163, COX1, was measured by qRT-PCR. * p<0.05, ** p<0.01 vs. vehicle.
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 the 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 high fat diet ($n = 7$). *$p<0.05$, **$p<0.01$. 
Figure 5. WISP1 expression in subjects with NASH

WISP1 mRNA expression in paired samples of human liver, SAT and VAT tissue (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 100nM insulin for 4h (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 the stimulation with 100nM insulin for 4h (n=3, B). WISP1 mRNA expression in SAT of non-diabetic subjects from the cohort IV before and after 4h of the hyperinsulinemic-euglycemic clamp (n=10, C) or hyperinsulinemic-hyperglycemic clamp (n=8, D). * p<0.05 vs. vehicle.
**Supplemental Figure 1. Differentiation of human mesenchymal stem cell derived adipocytes.**

(A) Expression of adipocyte markers PPARG and GLUT4 at different stages of differentiation of human mesenchymal stem cell (hMSC) derived adipocytes measured by real-time PCR ($n = 3$). (B) Oil Red O staining following adipocyte differentiation in the presence of vehicle (top) or 100ng/ml WISP1 (bottom). Representative phase-contrast photographs are shown. Differentiation of hMSC into adipocytes evidenced by the lipid accumulation in the cytosol of the cells (at least 80% of cells in each well). (C) Expression of WISP1 protein at different stages of adipocyte differentiation in the presence of vehicle or 100ng/ml WISP1. Representative western blots are shown. * $p<0.05$, ** $p<0.01$. 

---

**A**

<table>
<thead>
<tr>
<th></th>
<th>day 1</th>
<th>day 5</th>
<th>day 12</th>
<th>day 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPARG</td>
<td><img src="image1.png" alt="Graph" /></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GLUT4</td>
<td><img src="image2.png" alt="Graph" /></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**B**

- **Vehicle**
  - day 0
  - day 5
  - day 12
  - day 15
- **WISP1**
  - day 0
  - day 5
  - day 12
  - day 15

**C**

- **WISP1**
  - day 0
  - day 5
  - day 12
  - day 15
- **GAPDH**

---

Diabetes
Supplemental Figure 2. WISP1 effects on the insulin signaling

Western blots (A) and densitometric analysis of total and phosphorylated Akt protein levels (B) or total and phosphorylated ERK (44 and 42 kDa) protein levels (C) in extracts from 3T3-L1 adipocytes treated with 100 nM insulin for 30 min with or without pretreatment with 500ng/ml recombinant WISP1 protein for 30 min (n=3). * p<0.05, ** p<0.01.
### Supplemental Table 1. Primers used for real-time PCR

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Gene ID</th>
<th>Primer sequence forward</th>
<th>Primer sequence reverse</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Human</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCR7</td>
<td>1236</td>
<td>GGTGGTGACCTTGCCGTCATT</td>
<td>TGTCTGGGGATATCGTCCGGGA</td>
</tr>
<tr>
<td>CD136</td>
<td>4486</td>
<td>TTGGCAGCATTTTGAATGTG</td>
<td>AGGACAGGTGTGAGGACTGG</td>
</tr>
<tr>
<td>CD36</td>
<td>948</td>
<td>AGTCACTGAGCAATGTTGATG</td>
<td>CTGGAATCCTGGCTTCTTCA</td>
</tr>
<tr>
<td>COX1</td>
<td>4512</td>
<td>GCAACTGCTTTCTTCTTTTGC</td>
<td>TACTCACAGTGCCTCACCAC</td>
</tr>
<tr>
<td>COX2</td>
<td>4513</td>
<td>GCTGTTGGCTCTTGAGCCT</td>
<td>AATCAGGAGCTGCTTTTAC</td>
</tr>
<tr>
<td>FASN</td>
<td>2194</td>
<td>GCTCACTGACCCCTTCAGA</td>
<td>GGACCAGTGGAATGCA</td>
</tr>
<tr>
<td>GAPDH</td>
<td>2597</td>
<td>CCACATCGCTCTAGACATCA</td>
<td>CCGCCGGCGCAATACG</td>
</tr>
<tr>
<td>GLUT4</td>
<td>6517</td>
<td>GCGGAGCTTTGACGGCAAT</td>
<td>GGTGTGGCACTGTCTTCTCA</td>
</tr>
<tr>
<td>HPRT1</td>
<td>3251</td>
<td>TGACACTGGCAAAAACAATGCA</td>
<td>GGTTGGCTCTGTTCTTCA</td>
</tr>
<tr>
<td>IL10</td>
<td>3586</td>
<td>ACGGCTGCTGCTTGACATT</td>
<td>GGCATTTCCACCTGCTCCA</td>
</tr>
<tr>
<td>IL1B</td>
<td>3553</td>
<td>GCAATGAGATCACTTCTTCTTG</td>
<td>CAGAGGTCAGCTGGTCCAGA</td>
</tr>
<tr>
<td>IL6</td>
<td>3569</td>
<td>AGCCCTGAGAAGGAGACATGA</td>
<td>TCTGGCACGCTTCTTGGCTTCTT</td>
</tr>
<tr>
<td>MRC1</td>
<td>4360</td>
<td>CAGCGCTGTCATCGATT</td>
<td>GGCATTTCCACCTGCTCCA</td>
</tr>
<tr>
<td>PGC1A</td>
<td>10891</td>
<td>GCTACAGGAATATCGACCAAGA</td>
<td>ACACGGCGCTCTTCAATTG</td>
</tr>
<tr>
<td>PPARγ</td>
<td>5489</td>
<td>CAGGCGCTGTCATCGATT</td>
<td>GGCATTTCCACCTGCTCCA</td>
</tr>
<tr>
<td>RPLP0</td>
<td>6175</td>
<td>GCTGTTGGCTCTTCTTCTCA</td>
<td>TCTGGCACGCTTCTTGGCTTCTT</td>
</tr>
<tr>
<td>TNFα</td>
<td>7124</td>
<td>GGACCTGTTGGTACCGTGTTG</td>
<td>TCGAGAAGATGATGTGACTGCC</td>
</tr>
<tr>
<td>WISP1</td>
<td>8840</td>
<td>Hs_WISP1_1_SG QuantiTect primer assay, Cat Nr. QT00079492 (Qiagen, Germany)</td>
<td></td>
</tr>
<tr>
<td><strong>Mouse</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RPLP0</td>
<td>11837</td>
<td>CTGACCATCCAGACGGTTGTT</td>
<td>CCAGGAAGGACCTTACCTTCTT</td>
</tr>
<tr>
<td>WISP1</td>
<td>22402</td>
<td>Mm_Wisp1_1_SG QuantiTect primer assay, Cat Nr. QT00111230 (Qiagen, Germany)</td>
<td></td>
</tr>
</tbody>
</table>