




The novel adipokine WISP1 associates with insulin resistance and impairs insulin action in human myotubes and mouse hepatocytes

Tina Hörbelt^{1,2} · Christopher Tacke^{2,3,4} · Mariya Markova^{2,3} · Daniella Herzfeld de Wiza^{1,2} · Frederique Van de Velde⁵ · Marlies Bekaert⁵ · Yves Van Nieuwenhove⁶ · Silke Hornemann^{2,3} · Maria Rödiger^{2,7} · Nicole Seebeck^{2,3} · Elisabeth Friedl³ · Wenke Jonas^{2,7} · G. Hege Thoresen^{8,9} · Oliver Kuss^{2,10} · Anke Rosenthal¹¹ · Volker Lange^{12,13} · Andreas F. H. Pfeiffer^{2,3,4} · Annette Schürmann^{2,7} · Bruno Lapauw⁵ · Natalia Rudovich^{2,3,4,14} · Olga Pivovarova^{2,3,4} · D. Margriet Ouwers^{1,2,5} 

Received: 27 February 2018 / Accepted: 12 April 2018 / Published online: 12 May 2018
© Springer-Verlag GmbH Germany, part of Springer Nature 2018

Abstract

Aims/hypothesis Wingless-type (Wnt) inducible signalling pathway protein-1 (WISP1) has been recently identified as a proinflammatory adipokine. We examined whether WISP1 expression and circulating levels are altered in type 2 diabetes and whether WISP1 affects insulin signalling in muscle cells and hepatocytes.

Methods Serum and visceral adipose tissue (VAT) biopsies, for analysis of circulating WISP1 levels by ELISA and *WISP1* mRNA expression by real-time quantitative RT-PCR, were collected from normal-weight men (control group, $n = 33$) and obese men with ($n = 46$) and without type 2 diabetes ($n = 56$) undergoing surgery. Following incubation of primary human skeletal muscle cells (hSkMCs) and murine AML12 hepatocytes with WISP1 and insulin, insulin signalling was analysed by western blotting. The effect of WISP1 on insulin-stimulated glycogen synthesis and gluconeogenesis was investigated in hSkMCs and murine hepatocytes, respectively.

Results Circulating WISP1 levels were higher in obese men (independent of diabetes status) than in normal-weight men (mean [95% CI]: 70.8 [55.2, 86.4] ng/l vs 42.6 [28.5, 56.6] ng/l, respectively; $p < 0.05$). VAT *WISP1* expression was 1.9-fold higher in obese men vs normal-weight men ($p < 0.05$). Circulating WISP1 levels were positively associated with blood glucose in the OGTT and circulating haem oxygenase-1 and negatively associated with adiponectin levels. In hSkMCs and AML12

D. Margriet Ouwers and Olga Pivovarova contributed equally to the manuscript.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s00125-018-4636-9>) contains peer-reviewed but unedited supplementary material, which is available to authorised users.

✉ Olga Pivovarova
Olga.Pivovarova@dife.de

¹ Institute for Biochemistry and Pathobiochemistry, German Diabetes Center, Düsseldorf, Germany

² German Center for Diabetes Research (DZD), Muenchen-Neuherberg, Germany

³ Department of Clinical Nutrition, German Institute of Human Nutrition Potsdam-Rehbruecke, Arthur-Scheunert-Allee 114–116, 14558 Nuthetal, Germany

⁴ Department of Endocrinology, Diabetes and Nutrition, Charité University Medicine, Berlin, Germany

⁵ Department of Endocrinology, Ghent University Hospital, Ghent, Belgium

⁶ Department of Surgery, Ghent University Hospital, Ghent, Belgium

⁷ Department of Experimental Diabetology, German Institute of Human Nutrition, Potsdam, Germany

⁸ Department of Pharmaceutical Biosciences, School of Pharmacy, University of Oslo, Oslo, Norway

⁹ Department of Pharmacology, Institute of Clinical Medicine, University of Oslo, Oslo, Norway

¹⁰ Institute for Biometrics and Epidemiology, German Diabetes Center, Duesseldorf, Germany

¹¹ Clinic for Nutritional Medicine, Berlin, Germany

¹² Center for Obesity and Metabolic Surgery, Vivantes Hospital, Berlin, Germany

¹³ Helios Hospital Berlin-Buch, Berlin, Germany

¹⁴ Division of Endocrinology and Diabetology, Department of Internal Medicine, Spital Bülach, Bülach, Switzerland

Research in context

What is already known about this subject?

- Wnt-inducible signalling pathway protein-1 (WISP1) was recently identified as a proinflammatory adipokine
- WISP1 expression and secretion are associated with obesity and affected by diet-induced changes in body weight

What is the key question?

- Are WISP1 expression and circulating levels altered in type 2 diabetes and does WISP1 affect insulin signalling in muscle cells and hepatocytes?

What are the new findings?

- Circulating WISP1 levels and *WISP1* expression in visceral adipose tissue were increased in obesity independent of glycaemic status
- Circulating WISP1 levels were positively associated with blood glucose in an OGTT and with serum haem oxygenase-1 and negatively associated with adiponectin
- WISP1 impaired insulin signalling in primary human skeletal muscle cells and murine hepatocytes

How might this impact on clinical practice in the foreseeable future?

- WISP1 may regulate whole-body insulin sensitivity owing to its effects on insulin signalling in liver and muscle and is a promising target for prevention and treatment of obesity and diabetes

hepatocytes, recombinant WISP1 impaired insulin action by inhibiting phosphorylation of insulin receptor, Akt and its substrates glycogen synthase kinase 3 β , FOXO1 and p70S6 kinase, and inhibiting insulin-stimulated glycogen synthesis and suppression of gluconeogenic genes.

Conclusions/interpretation Circulating WISP1 levels and *WISP1* expression in VAT are increased in obesity independent of glycaemic status. Furthermore, WISP1 impaired insulin signalling in muscle and liver cells.

Keywords Adipokine · Akt · Insulin action · Insulin resistance · Type 2 diabetes · Visceral adipose tissue · WISP1

Abbreviations

CRP	C-reactive protein
FOXO1	Forkhead box O1
GSK3 β	Glycogen synthase kinase 3 β
HO-1	Haem oxygenase-1
hSkMC	Human skeletal muscle cell
IR β	Insulin receptor β
LRP	LDL receptor-related protein
MCP-1	Monocyte chemotactic protein 1
p70S6K	p70 S6 kinase
SAT	Subcutaneous adipose tissue
sFRP	Secreted frizzled-related protein
VAT	Visceral adipose tissue
Wnt	Wingless-type
WISP1	Wnt-inducible signalling protein-1

Introduction

The wingless-type (Wnt) signalling pathway is closely associated with the pathophysiology of cancers and has been linked to adipogenesis and hypertrophic obesity [1, 2]. Regulation of

the Wnt signalling system is complex and involves multiple components, including 19 different Wnt ligands that can bind to a family of ten distinct frizzled receptors and seven families of co-receptors, the most well-known being the LDL receptor-related proteins (LRPs) 5 and 6 [3]. Numerous secreted proteins that antagonise Wnt signalling by preventing ligand–receptor interactions have been identified, including members of the secreted frizzled-related protein (sFRP) and Dickkopf families [4]. Depending on the ligand–receptor combination, activation of Wnt signalling pathways results in the activation of either the canonical or the less-well-characterised non-canonical Wnt pathway [5, 6]. In the canonical pathway, activation of the frizzled receptors and LRP5/6 leads to inhibition of the activity of cytosolic glycogen synthase kinase 3 β (GSK3 β) [7, 8]. This results in stabilisation of the transcriptional regulator β -catenin and induction of the expression of Wnt target genes [5, 6]. Importantly, in vitro studies on pre-adipocytes report an association between alterations in Wnt and LRP5 activity and the regulation of the insulin-mediated phosphorylation of Akt and its substrate GSK3 β , suggesting that changes in the activity of Wnt signalling may participate in the pathophysiology of insulin resistance and type 2 diabetes [9].

Adipocytes have been found to express and/or release many targets and regulators of the Wnt signalling pathway, including Wnt-inducible signalling protein (WISP) 2, sFRP1, sFRP2, sFRP4 and sFRP5 [10–12]. Moreover, expression and release of these proteins is altered in obesity [10–12]. Recently, we identified WISP1 (also known as CCN4) as an adipokine in human and murine adipose tissue [13]. Cross-sectional observations in glucose-tolerant humans have demonstrated that *WISP1* mRNA levels in adipose tissue are positively associated with fasting insulin levels and macrophage infiltration in adipose tissue and are associated negatively with insulin sensitivity, as measured by hyperinsulinaemic–euglycaemic clamp [13]. Furthermore, *WISP1* expression is affected by diet-induced changes in body weight [13]. In humans, weight loss led to a reduction in *WISP1* expression, whereas feeding mice a high-fat diet increased the expression of WISP1 in adipose tissue [13]. A study in women with gestational diabetes and insulin resistance showed increased circulating levels of WISP1 and identified BMI and insulin resistance as determinants of WISP1 levels [14]. Another very recently published study observed a direct relationship between circulating WISP1 levels and visceral adiposity and signs of insulin resistance, independently of glucose tolerance [15].

Despite these observations, data on the potential relevance of WISP1 in the pathophysiology of insulin resistance and type 2 diabetes are limited. This study examined whether circulating levels of WISP1 and its expression in visceral adipose tissue (VAT) are altered in obese men with or without type 2 diabetes vs normal-weight men. Furthermore, we investigated in vitro whether WISP1 impairs insulin action in primary human skeletal muscle cells (hSkMCs) and murine AML12 hepatocytes.

Methods

Participant characteristics We recruited 33 normal-weight men and 102 obese men, of whom 46 had type 2 diabetes according to the American Diabetes Association criteria [16], from the Obster [17] and HepObster [18] cohort. Obese men were scheduled for bariatric surgery, whereas normal-weight men were scheduled for elective surgery in the abdominal region. On the day of surgery, a blood sample was collected after overnight fasting. During surgery, VAT and subcutaneous adipose tissue (SAT) biopsies were collected. In a subcohort of obese men ($n = 42$), an OGTT was performed using 75 g glucose. The study protocol was approved by the Ethics Committee of Ghent University Hospital and conducted according to the principles of the Declaration of Helsinki. All participants gave their written informed consent. See electronic supplementary material (ESM) [Methods](#) for more details. Samples collected from the participants were not randomised and no blinding regarding group assignment was applied.

Serum analysis Serum WISP1 levels were determined using a Duoset human WISP-1/CCN4 ELISA assay (R&D systems, Wiesbaden, Germany). C-reactive protein (CRP), insulin, adiponectin, chemerin, monocyte chemoattractant protein 1 (MCP-1), leptin, omentin and haem oxygenase-1 (HO-1) serum levels were quantified using commercial assays as described in ESM [Methods](#).

Determination of adipocyte cell size VAT and SAT biopsies were used to assess adipocyte cell size by hematoxylin–eosin staining of VAT and SAT paraffin slides. See ESM [Methods](#) for details.

Culture of adipose tissue explants Adipose tissue explants were obtained from obese individuals and cultured in DMEM/F12 (Gibco, Darmstadt, Germany). See ESM [Methods](#) for details.

Cell culture hSkMCs were differentiated from proliferating satellite cells and were cultured as previously described [19]. The mouse AML12 (alpha mouse liver 12) hepatocyte cell line was maintained in DMEM/F12 (Thermo Fisher Scientific, Waltham, MA, USA) for 48 h. Primary hepatocytes were isolated from male C57BL/6 mice as described [20, 21]. 3T3-L1 fibroblasts were differentiated to adipocytes in Iscove's Modified Dulbecco's Medium IMDM, PAN Biotech, Aidenbach, Germany). See ESM [Methods](#) for details.

Cell treatment with recombinant WISP1 Myotubes and primary hepatocytes were serum-starved and AML12 cells were cultured in medium without insulin and serum for 4 h followed by 24 h exposure to recombinant WISP1 prior to insulin stimulation. See ESM [Methods](#) for details.

Knockdown of WISP1 in 3T3-L1 adipocytes Before initiating differentiation into adipocytes, 3T3-L1 cells were transfected with siRNA for *WISP1* or scrambled siRNA (used as a control). See ESM [Methods](#) for details.

Gene expression analysis Real-time quantitative RT-PCR using specific primers (ESM Table 1) was performed to determine expression levels of mRNAs for *WISP1*, *CCL2* (encoding MCP-1), *RARRES2* (encoding chemerin), *ITLN1* (encoding omentin), *HMOX1* (encoding HO-1), *RPS18*, *UBE2D2*, *YWHAZ* and *RPLP0* in human adipose tissue and *Wisp1*, *Il6*, *Tnf*, *Ccl2*, *G6pc*, *Pck*, *Rplp0* and *18s* in mouse 3T3-L1 adipocytes or primary hepatocytes. See ESM [Methods](#) and ESM Table 1 for details.

Analysis of protein phosphorylation and abundance Analysis of insulin signalling was performed in hSkMCs and AML12 lysates by western blotting with antibodies recognising phosphorylated and/or non-phosphorylated Akt, glycogen

synthase kinase, forkhead box O1 (FOXO1), p70S6kinase (p70S6K), insulin receptor β (IR β), IRS1 and IRS2, α -tubulin and GAPDH all used as per manufacturer's instructions and as described in ESM [Methods](#).

Analysis of glycogen synthesis in primary hSkMCs Glycogen synthesis in hSkMCs was assessed by the incorporation of D-[U-¹⁴C]glucose into glycogen in the absence or presence of insulin (100 nmol/l). See ESM [Methods](#) for details.

Statistical analysis Statistical analysis of the clinical data and cell culture experiments was performed using SPSS Statistics (version 24.0; IBM, Armonk, NY, USA) and GraphPad Prism (version 7.0; GraphPad Software, La Jolla, CA, USA), respectively. The data are expressed as mean (95% CI) unless stated otherwise. Differences between groups were calculated by ANOVA followed by a Bonferroni correction for multiple comparisons. Gutt index of insulin sensitivity was calculated as described [22]. Linear regression analysis with adjustments for age and/or BMI was applied to identify correlates of *WISP1* mRNA expression in VAT as well as circulating *WISP1* levels and determinants of adipose tissue and glucose metabolism. In the correlation and regression analysis of glucose in OGTT, HOMA-IR and Gutt index, individuals treated with insulin (inclusive of all individuals with diabetes) were excluded. Variables showing a skewed distribution were log-transformed prior to analysis. In all analyses, a *p* value of <0.05 was considered as statistically significant.

Results

Participant cohort The characteristics of the 33 normal-weight men and 102 obese men, of whom 46 had type 2 diabetes, are listed in Table 1. The obese men without type 2 diabetes had a higher BMI, fat mass, and larger adipocytes than normal-weight men (all *p* < 0.001), increased fasting insulin levels (*p* < 0.01), a reduced insulin sensitivity (*p* < 0.05) and increased beta cell function (*p* < 0.001), both estimated by HOMA modelling (*p* < 0.05). The obese participants with type 2 diabetes were older than the obese men without type 2 diabetes (*p* < 0.001). In addition, fat mass (*p* < 0.05) and fasting glucose levels (*p* < 0.001) were higher, and beta cell function (*p* < 0.05) was reduced in the obese men with type 2 diabetes vs those without type 2 diabetes. A subgroup of 42 obese men, of whom eight had type 2 diabetes, underwent an OGTT. As expected, the post-load glucose levels were higher in individuals with type 2 diabetes (ESM Fig. 1a, b), while post-load insulin levels were lower (ESM Fig. 1c, d), when compared with participants without type 2 diabetes.

WISP1 serum levels and gene expression in VAT in obesity and type 2 diabetes *WISP1* circulating levels were obtained

in 123 out of 135 participants. In samples from two participants (one normal weight and one obese), *WISP1* levels were below the limit of detection; in the other participants without data, no serum sample was available for analysis. The circulating *WISP1* level was 42.6 (28.5, 56.6) ng/l in normal-weight men (*n* = 30) and 70.8 (55.2, 86.4) ng/l in obese men (*n* = 93) (*p* < 0.05, Fig. 1a). There was no statistically significant difference in circulating *WISP1* level between obese men with (69.4 [48.5, 90.3] ng/l; *n* = 44) and without type 2 diabetes (72.2 [48.4, 95.6] ng/l; *n* = 49) (Fig. 1a).

VAT biopsies to analyse *WISP1* gene expression levels were available for 92 participants from the 'HepObster' cohort. The expression of *WISP1* was 1.9-fold higher in VAT from obese men (*n* = 76) than in VAT from normal-weight men (*n* = 16) (*p* < 0.05). As with circulating *WISP1* levels, we observed no statistically significant differences in *WISP1* expression levels between obese men with (*n* = 31) and without type 2 diabetes (*n* = 45) (Fig. 1b). In paired SAT samples, *WISP1* mRNA levels were very low; reliable detection of *WISP1* (C_t < 35 and single-peak melting curve) was achieved in only nine of the 33 samples analysed (data not shown). In addition, we compared *WISP1* mRNA expression levels in the explants of paired SAT and VAT samples collected from study participants with obesity (four men, two women, BMI 47.94 \pm 3.96 kg/m², age 43.73 \pm 4.59 years). In accordance with our previous report [13], *WISP1* expression was ninefold higher in VAT than in SAT (*p* < 0.05) (Fig. 1c). Therefore, only values of the *WISP1* expression in VAT were used for further analysis.

Correlation of WISP1 serum levels and WISP1 VAT gene expression with determinants of body composition and glucose metabolism *WISP1* VAT expression was positively associated with HOMA-IR (*r* = 0.272, *p* = 0.034) and with fasting glucose levels (*r* = 0.262, *p* = 0.042) (ESM Table 2). Furthermore, a positive association with fasting insulin levels was observed (*r* = 0.231), although this did not reach statistical significance (*p* = 0.073). Adjusting for age had no major impact on the relationship between *WISP1* gene expression and HOMA-IR (β = 0.261, *p* = 0.041) but the associations with fasting glucose (β = 0.232, *p* = 0.085) and fasting insulin (β = 0.233, *p* = 0.069) lost their statistical significance.

Circulating *WISP1* levels displayed a negative association with age (*r* = -0.193, *p* = 0.034) and positive associations with determinants of body composition (e.g. BMI [*r* = 0.207, *p* = 0.022]) as well as with subcutaneous adipocyte cell size (*r* = 0.368, *p* = 0.015) (ESM Table 2). There was also an association between circulating *WISP1* level and visceral adipocyte cell size (*r* = 0.292, *p* = 0.058), although this did not reach statistical significance. Several determinants of glucose metabolism were positively associated with serum *WISP1* levels: fasting insulin (*r* = 0.255, *p* = 0.022) and glucose (*r* = 0.188, *p* = 0.094); HOMA-IR (*r* = 0.275, *p* = 0.014); post-load glucose levels at multiple time points (30 min [*r* = 0.312, *p* =

Table 1 Participant characteristics

Variable	Normal weight	Obese	Obese + type 2 diabetes	<i>p</i> value
<i>n</i>	33	56	46	
Age, years	46.9 (42.3, 51.5)	42.1 (39.2, 45.1)	52.0 (49.2, 54.8) ^{†††}	<0.001
BMI, kg/m ²	24.2 (23.2, 25.2)	41.2 (39.7, 42.6) ^{***}	43.1 (41.1, 45.1) ^{***}	<0.001
Fat, % body weight	24.2 (22.1, 26.4)	37.6 (33.5, 41.7) ^{***}	44.1 (41.3, 46.9) ^{***,†}	<0.001
SAT cell size, μm ²	3508 (2724, 4292)	6425 (5726, 7123) ^{***}	5765 (5237, 6294) ^{***}	<0.001
VAT cell size, μm ²	3494 (2679, 4309)	6004 (5027, 6982) ^{***}	5576 (4899, 6253) ^{***}	<0.001
Fasting glucose, mmol/l	5.06 (4.67, 5.45)	5.67 (5.21, 6.13)	8.09 (7.18, 9.01) ^{***,†††}	<0.001
Fasting insulin, pmol/l	37.4 (30.0, 44.7)	136 (90.3, 181) ^{**}	170 (135, 204) ^{***}	<0.001
HOMA-IR	1.20 (0.94, 1.47)	5.79 (2.94, 8.63) [*]	8.85 (6.86, 10.84) ^{**}	<0.001
HOMA2-%B	81.8 (64.1, 100)	139 (118, 160) ^{***}	106 (87.3, 124) [†]	<0.001
HbA _{1c} , mmol/mol ^a	NA	39.67 (36.28, 43.06)	58.25 (47.65, 68.85) ^{††}	
HbA _{1c} , % ^a	NA	5.78 (5.47, 6.09)	7.48 (6.51, 8.45) ^{††}	
Adiponectin, mg/l	8.86 (7.35, 10.38)	32.04 (24.71, 39.37) ^{***}	34.94 (29.66, 40.23) ^{***}	<0.001
CRP, nmol/l	22 (10.29, 33.71)	45.52 (34.00, 57.05) [*]	35.62 (24.95, 46.38)	
CRP, mg/l	2.31 (1.08, 3.54)	4.78 (3.57, 5.99) [*]	3.74 (2.62, 4.87)	<0.01
Chemerin, μg/l	188 (162.9, 214.8)	206.70 (188, 225.4)	235 (213.1, 256.9) [*]	<0.05
Leptin, μg/l	3.74 (2.41, 5.06)	32.04 (24.71, 39.37) ^{***}	34.94 (29.66, 40.23) ^{***}	<0.001
MCP-1, ng/l	220 (338, 499.1)	334.9 (300.2, 369.6)	408.1 (358.3, 457.9)	<0.05
Omentin, μg/l	383.5 (349.5, 417.6)	387.6 (348.8, 426.5)	442.8 (400.6, 485)	>0.05
HO-1, ng/l	555.0 (294.2, 815.8)	867.1 (563.5, 1170.7)	578.4 (344.4, 812.3)	0.072
VAT gene expression levels, AU				
<i>ADIPOQ</i>	171.7 (61.8, 281.6)	43.6 (32.9, 54.2) ^{**}	54.6 (32.9, 76.3) ^{***,††}	<0.01
<i>RARRES2</i>	1.16 (0.87, 1.45)	0.92 (0.82, 1.02)	0.81 (0.70, 0.93) [*]	<0.05
<i>CCL2</i>	3.09 (0.90, 5.28)	1.463 (0.83, 2.10)	1.39 (0.66, 2.12)	>0.05
<i>ITLN1</i>	9.04 (−0.60, 18.68)	13.86 (7.7, 20.03)	16.56 (9.07, 24.04)	>0.05
<i>HMOX1</i> ^b	1.75 (0.95, 2.55)	1.96 (−0.29, 4.22)	2.17 (1.18, 3.16)	0.791

The data are presented as mean (95% CI)

Differences between the participant groups were calculated using ANOVA and Bonferroni correction for multiple comparisons

p* < 0.05, *p* < 0.01 and ****p* < 0.001 obese men vs normal-weight men; †*p* < 0.05, ††*p* < 0.01 and †††*p* < 0.001, obese men with vs without type 2 diabetes. The *p* values in the right hand column show the corresponding significance across all three groups by ANOVA

^a For HbA_{1c}, only data for ten obese participants and 15 participants with type 2 diabetes available

^b For *HMOX1* expression, data for 15 normal-weight individuals, five obese individuals and 16 individuals with type 2 diabetes available

HOMA2-%B, computer model of homeostasis model assessment (HOMA) for β-cell function (%B)

0.072] 60 min [*r* = 0.443, *p* = 0.009], 120 min [*r* = 0.335, *p* = 0.052]) and AUC for glucose between 0 and 120 min (*r* = 0.413, *p* = 0.015) (ESM Table 2). The associations with BMI (β = 0.192, *p* = 0.033), subcutaneous adipocyte size (β = 0.322, *p* = 0.039), post-load glucose at 60 min (β = 0.442, *p* = 0.013) and AUC glucose_{0–120min} (β = 0.428, *p* = 0.021) remained significant after adjusting for age (Table 2). After adjusting for age and BMI, the associations between circulating WISP1 and post-load glucose at 60 min (β = 0.434, *p* = 0.018) and AUC glucose_{0–120min} (β = 0.420, *p* = 0.028) were still statistically significant (Table 2). Moreover, circulating WISP1 levels were negatively associated with Gutt's index of insulin sensitivity (*r* = −0.401, *p* = 0.035; *n* = 28; ESM Table 2) and this association remained significant after adjusting for age and BMI (β = −0.438, *p* = 0.037; Table 2).

As shown in ESM Table 3, the association between HOMA-IR and *WISP1* gene expression lost statistical significance upon adjustment for age and BMI (β = 0.240, *p* = 0.101).

Correlation of WISP1 serum and gene expression levels with markers of adipose tissue and systemic inflammation

Because WISP1 has been shown to induce an inflammatory response in human macrophages [13], we additionally investigated the association of *WISP1* expression and serum levels with markers of adipose tissue inflammation (mRNA levels of *ADIPOQ*, *RARRES2*, *CCL2*, *ITLN1*) and systemic inflammation (adiponectin, CRP, chemerin, leptin, MCP-1, omentin). Moreover, we investigated the association of WISP1 with serum HO-1 which is associated with insulin resistance and adipose tissue inflammation and activates Wnt signalling

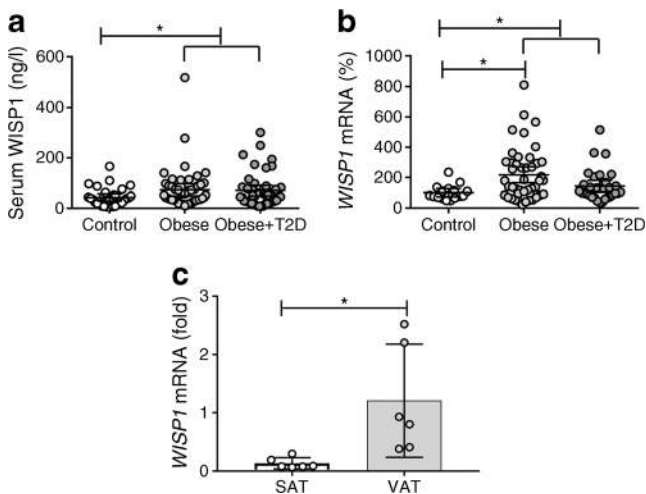


Fig. 1 WISP1 serum levels and gene expression in VAT. (a, b) Quantification of circulating WISP1 levels (a) and gene expression in VAT (b) from normal-weight (control, $n = 30$ [a], $n = 16$ [b]) and obese men with ($n = 44$ [a], $n = 31$ [b]) and without ($n = 49$ [a], $n = 45$ [b]) type 2 diabetes. (c) Expression of *WISP1* in paired samples of VAT and SAT (c, $n = 6$). Data (a–c) are presented as dot plot in which the mean and 95% CI for each group are shown. Differences among normal-weight men and obese men with and without T2D (brackets) were analysed by Mann–Whitney *U* test (a, b). Brackets link data for obese men without and with T2D for analysis. Differences among all groups were analysed by ANOVA and Bonferroni correction for multiple comparisons (b). Data were analysed by paired Student's *t* test (c). * $p < 0.05$, obese vs normal-weight men (a, b); * $p < 0.05$, VAT vs SAT (c). T2D, type 2 diabetes

[23, 24]. Compared with normal-weight participants, circulating levels of adiponectin, leptin and CRP were increased and VAT *ADIPOQ* mRNA levels were decreased in obese individuals ($p < 0.001$, $p < 0.001$, $p = 0.014$ and $p = 0.002$, respectively)

Table 2 Regression analysis of circulating WISP1 levels

Variable	Unadjusted	Age	Age + BMI
BMI, kg/m ²	0.207 (0.022)*	0.192 (0.033)*	NA
Fat, % body weight	0.279 (0.067)	0.288 (0.056)	−0.115 (0.794)
SAT cell size, μm ²	0.368 (0.015)*	0.322 (0.039)*	0.191 (0.399)
VAT cell size, μm ²	0.292 (0.058)	0.265 (0.081)	0.146 (0.397)
Fasting glucose, mmol/l ^a	0.202 (0.091)	0.201 (0.079)	0.183 (0.114)
Fasting insulin, pmol/l ^a	0.241 (0.044)*	0.182 (0.123)	0.128 (0.385)
HOMA-IR ^{a,b}	0.262 (0.028)*	0.210 (0.073)	0.171 (0.231)
HOMA2-%B ^{a,b}	0.099 (0.415)	0.035 (0.771)	−0.056 (0.676)
ISI Gutt _{0–120 min} ^b	−0.401 (0.035)*	−0.397 (0.042)*	−0.438 (0.037)*
Glucose, OGTT at 30 min ^{a,b}	0.312 (0.072)	0.312 (0.107)	0.313 (0.108)
Glucose, OGTT, at 60 min ^{a,b}	0.443 (0.009)**	0.442 (0.013)*	0.434 (0.018)*
Glucose, OGTT, at 120 min ^{a,b}	0.335 (0.052)	0.331 (0.077)	0.320 (0.100)
AUC glucose _{0–20 min} , mmol/l ^{a,b}	0.416 (0.015)*	0.428 (0.021)*	0.420 (0.028)*

The data indicate standardised regression coefficient β , with p value shown in parentheses using measured WISP1 serum data of study participants of all groups (normal-weight, obese, obese+T2D) combined

^a Variables with a skewed distribution were log-transformed prior to the regression analysis

^b Participants treated with insulin were excluded from the analysis

* $p < 0.05$ and ** $p < 0.01$

ISI, insulin sensitivity index

(Table 1). Serum levels of chemerin and VAT levels of *RARRES2* mRNA were higher in obese individuals with or without diabetes ($p = 0.014$ and $p = 0.010$, respectively).

Circulating WISP1 levels showed a negative association with serum adiponectin ($r = -0.190$, $p = 0.031$) and a highly significant positive association with serum HO-1 levels ($r = 0.437$, $p = 1.3 \times 10^{-5}$) (ESM Table 4). The association with serum adiponectin remained significant after adjusting for age ($\beta = -0.176$, $p = 0.041$); the association with HO-1 remained significant after adjusting for age ($\beta = 0.319$, $p = 0.002$) and for age and BMI ($\beta = 0.303$, $p = 0.004$). *WISP1* mRNA expression in VAT showed a positive correlation with *CCL2* expression ($r = 0.253$, $p = 0.021$) (ESM Table 4) and this association remained statistically significant after adjusting for age and BMI ($\beta = 0.266$, $p = 0.015$).

WISP1 silencing with siRNA in 3T3-L1 adipocytes had no effect on mRNA expression of *Il6*, *Tnf* and *Ccl2* (ESM Fig. 2).

Effects of WISP1 on insulin signalling The observed relationship between circulating WISP1 and HOMA-IR as well as post-load glucose levels, together with our previous observation of the negative association between *WISP1* VAT expression and hyperinsulinaemic–euglycaemic clamp-derived insulin sensitivity [13], led us to test the hypothesis that WISP1 may interfere with insulin signalling. To examine this in more detail, we exposed primary hSkMCs to recombinant WISP1 before analysis of insulin action. As shown in Fig. 2 a, b, the insulin-mediated induction of Akt on Thr308 and Ser473 phosphorylation in primary hSkMCs was reduced by 40% in cells that were exposed to 1 μg/l WISP1 before the addition of

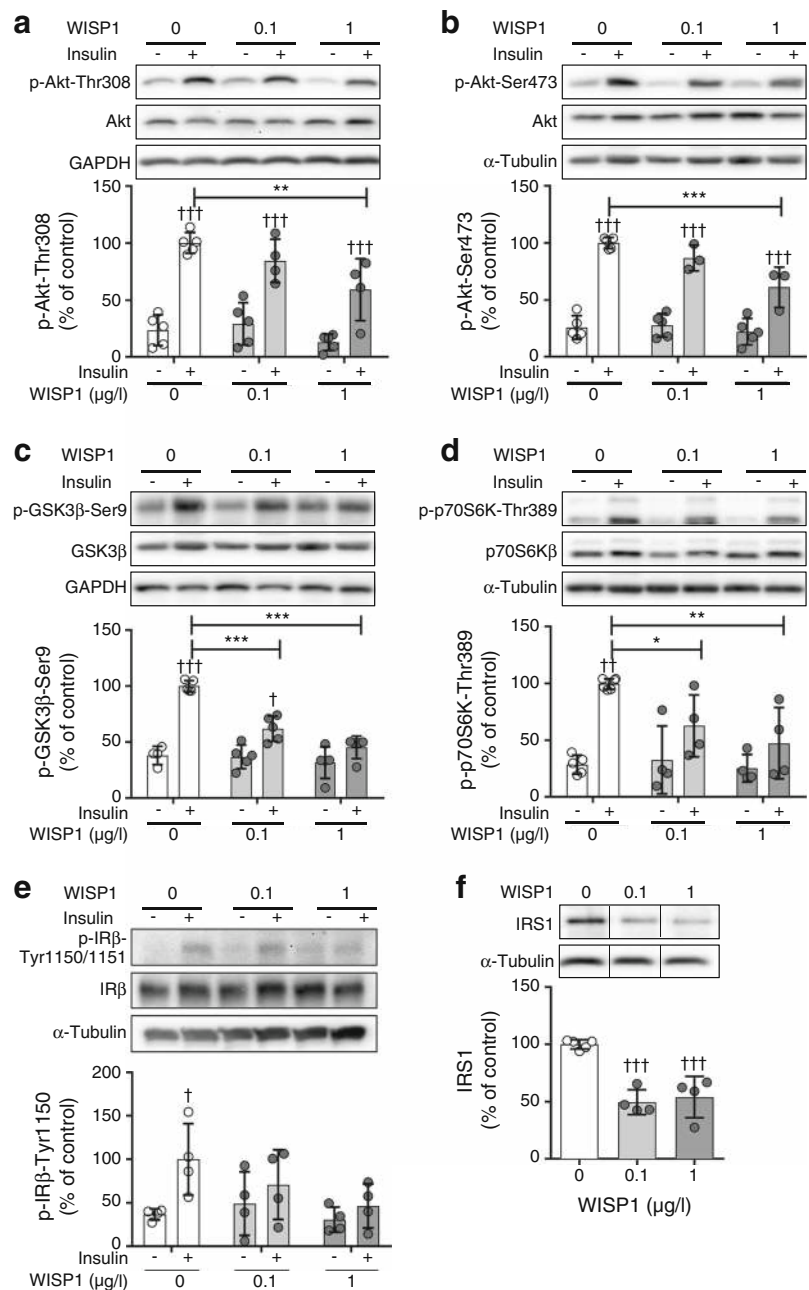
insulin. The reductions in insulin-mediated Akt phosphorylation induced by WISP1 were accompanied by decreases in insulin-mediated GSK3 β -Ser9, p70S6K-Thr389 and IR β -Tyr-1150/1151 phosphorylation (Fig. 2c–e). This inhibition of the insulin-mediated phosphorylation was achieved already in the presence of 0.1 μ g/l WISP1. Of further note, the reductions in phosphorylation levels could not be ascribed to changes in the protein abundances of Akt, IR β , GSK3 β or p70S6K (ESM Fig. 3). In contrast, the abundance of IRS1 protein was reduced by ~50% in cells that were exposed to WISP1 (Fig. 2f).

To demonstrate the functional relevance of the reduced insulin signalling, we further investigated effects of the WISP1

treatment on insulin-stimulated glycogen synthesis. Insulin treatment (100 nmol/l) for 3 h significantly increased glycogen synthesis in primary human myotubes compared with basal conditions, whereas in cells pre-incubated with 0.1 or 1 μ g/l WISP1 for 24 h, this effect was completely abrogated (Fig. 3).

Largely comparable data were obtained for the murine hepatocyte cell line AML12. Exposing AML12 cells to WISP1 caused a dose-dependent reduction in insulin-induced Akt-Thr308 and Akt-Ser473 phosphorylation (Fig. 4a, b). The induction of GSK3 β -Ser9 phosphorylation by insulin in hepatocytes was not affected by WISP1 (Fig. 4c) whereas the inhibition of insulin-induced Akt phosphorylation was accompanied

Fig. 2 Effect of WISP1 on insulin signalling in primary hSkMCs. Representative western blots and bar graphs show the effects of WISP1 on insulin-stimulated phosphorylation of Akt-Thr308 (a), Akt-Ser473 (b) GSK3 β -Ser9 (c), p70S6 kinase (K)-Thr389 (d) and IR β -Tyr1150/1151 (e) and on protein abundance of IRS1 (f). The dividing lines in the blots in (f) indicate places where the blot has been cut. The scattered bar graphs indicate the mean \pm SD for the phosphorylation levels obtained in three to five independent experiments in primary hSkMCs obtained from different donors. The phosphorylation levels were normalised for the protein abundances of the non-phosphorylated proteins, GAPDH or α -tubulin, as indicated. The values obtained in cells incubated with 100 nmol/l insulin only were considered as the control and set at 100%. The effects of WISP1 and insulin were analysed by two-way ANOVA with Bonferroni correction for multiple comparisons. * p < 0.05, ** p < 0.01 and *** p < 0.001, with vs without WISP1 incubation; † p < 0.05, †† p < 0.01 and ††† p < 0.001, with vs without insulin stimulation



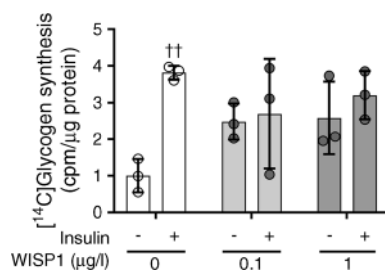


Fig. 3 Effect of WISP1 on insulin-stimulated glycogen synthesis in primary hSkMCs. Myotubes were exposed to WISP1 for 24 h before glycogen synthesis was determined as incorporation of D-[U- 14 C]glucose into glycogen in the absence or presence of insulin (100 nmol/l) for 3 h. Data are normalised to amount of protein for three independent experiments ($n = 3$) and are shown as mean \pm SD. The effects of WISP1 and insulin were analysed by two-way ANOVA and Bonferroni correction for multiple comparisons. $^{\dagger\dagger}p < 0.01$, with vs without insulin

by a reduction in p70S6K-Thr389 and IR β -Tyr-1150/1151 phosphorylation (Fig. 4d, e). Therefore, to corroborate the effects of WISP1 on distal Akt signalling, we analysed another Akt substrate, namely phosphorylation of FOXO1 at Ser256. Figure 4f shows that incubating AML12 cells with 0.1 and 1 μ g/l WISP1 impaired the induction of FOXO1-Ser256 phosphorylation by insulin. In contrast to the findings in myotubes, the abundance of IRS1 protein was doubled in AML12 cells exposed to WISP1 (Fig. 4g), whereas the abundances of Akt, GSK3 β , P70S6K, IR β and FOXO1 were not affected (ESM Fig. 4). In addition, WISP1 incubation had no effect on the abundance of IRS2, the preferred insulin receptor substrate in the liver [25]. Further, WISP1 treatment abrogated the insulin-mediated suppression of the gluconeogenic genes *Pck1* and *G6pc* in mouse primary hepatocytes (Fig. 5).

Discussion

Our study showed that both circulating levels of WISP1 and expression of *WISP1* mRNA in VAT are increased in obese men, irrespective of type 2 diabetes status. Regression analysis showed that *WISP1* expression and circulating levels of WISP1 were associated with variables reflecting insulin resistance and adipose tissue inflammation, even after adjusting for age and BMI. In vitro studies on primary hSkMCs as well as the mouse hepatocyte cell line AML12 showed that recombinant WISP1 directly impaired insulin action by inhibiting the Akt signalling pathway.

The increased *WISP1* expression in VAT of obese men is in line with previous reports from our group and others [13–15, 26] suggesting that *WISP1* expression is regulated by body weight. High-fat feeding in mice increased the expression of *WISP1* in adipose tissue [13, 27] while diet-induced weight loss in humans lowered *WISP1* expression in adipose tissue [13]. The present study also detailed the relationship between WISP1 and insulin sensitivity. In individuals with normal

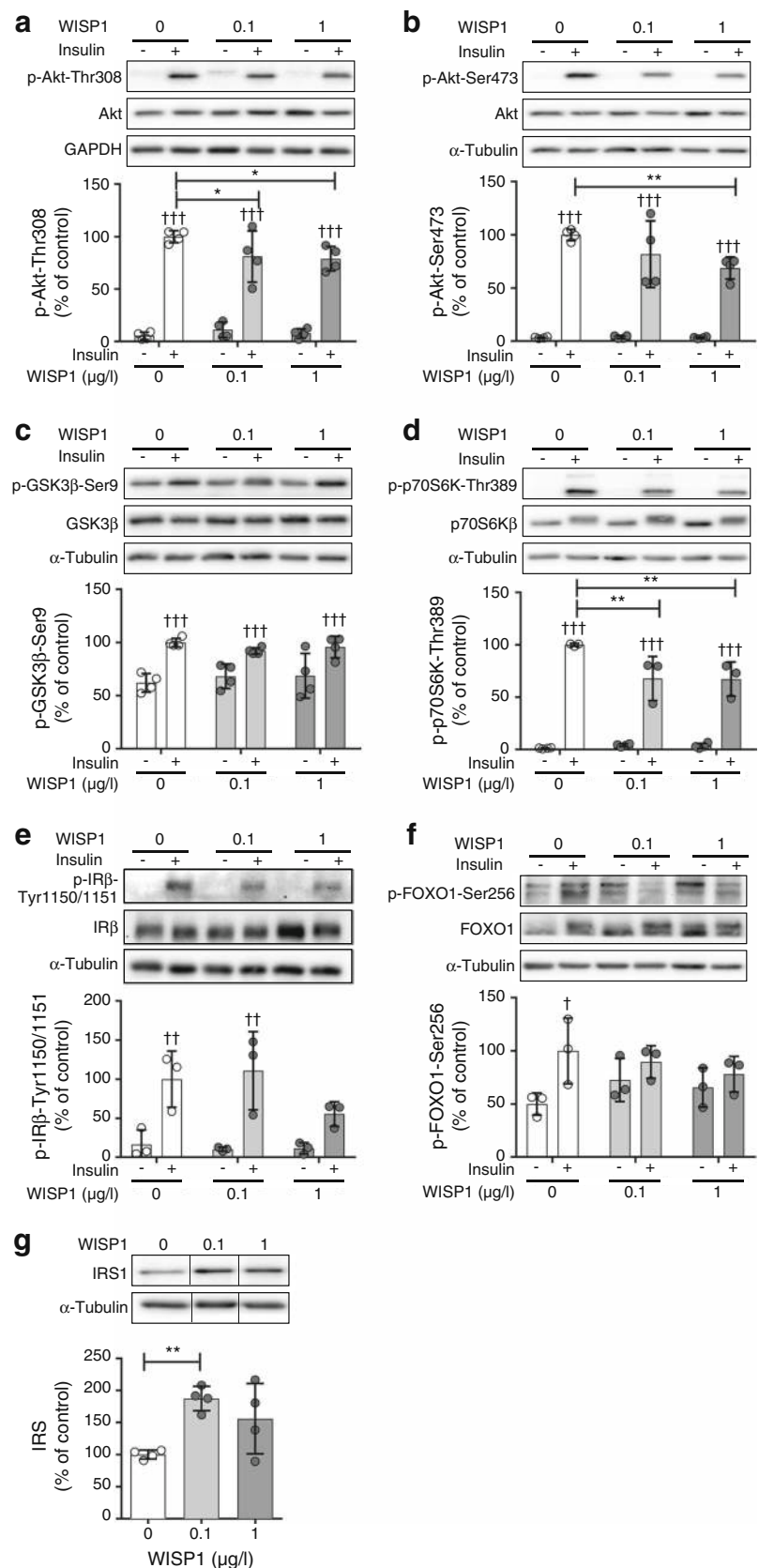
glucose tolerance, *WISP1* expression in adipose tissue is related to determinants of insulin sensitivity [13, 26]. Here, we additionally showed that circulating levels of WISP1 are negatively associated with insulin sensitivity. Although the associations with fasting glucose and insulin levels as well as HOMA-IR in this cohort of obese men were confounded by BMI, this was not the case when considering glucose clearance after an OGTT. The association between circulating WISP1 and post-load glucose levels was not affected by adjustments for age and BMI, strongly suggesting that WISP1 may interfere with insulin signalling in target tissues for insulin action.

Another interesting finding is that circulating WISP1 level is positively associated with plasma glucose in the OGTT (independent of BMI) and negatively with the Gutt index of insulin sensitivity, confirming the association of WISP1 with insulin resistance. However, circulating WISP1 is not further increased in obese individuals with diabetes and shows no association with HOMA-IR after adjustment for BMI. It should be noted that in the analysis of glucose in the OGTT, HOMA-IR and Gutt index, individuals treated with insulin were excluded from the analysis. In our cohort, all individuals with diabetes were treated with insulin, so that all those with diabetes were completely excluded from the analysis. For the correct analysis of WISP1 association with plasma glucose in future studies, drug-naive individuals with diabetes should be investigated. Nevertheless, our results confirm that WISP1 level is directly related to adiposity independent of glycaemic status. This is in agreement with previously published data of Barchetta et al [15] and Tacke et al [26], who also found no difference in plasma WISP1 levels between diabetic and non-diabetic individuals.

The in vitro data reported in this study corroborate the suggestion that WISP1 may interfere with insulin signalling in insulin target tissues (Fig. 6). Recombinant WISP1 was found to impair insulin signalling in two different cell types, namely primary hSkMCs and murine AML12 hepatocytes. In both cell types, WISP1 inhibited the insulin-mediated phosphorylation of Akt, an important regulator of multiple aspects of glucose metabolism, such as glucose uptake, glycogen synthesis and suppression of hepatic glucose production by insulin. Moreover, we were able to demonstrate the functional relevance of the reduced insulin signalling in both cell types. Human myotubes pre-incubated with WISP1 displayed impaired insulin-stimulated glycogen synthesis and exposure to WISP1 abrogated the insulin-mediated suppression of the gluconeogenic genes *Pck1* and *G6pc* in mouse primary hepatocytes. These in vitro observations should be validated in further animal studies in vivo.

WISP1 deficiency in mice leads to a slightly lower body weight than found in wild-type animals but unfortunately no data on glucose traits or insulin sensitivity are available yet [28]. In contrast, WISP1 did not interfere with insulin-mediated Akt phosphorylation in our previous study on 3T3L1 adipocytes [13]. Apart from potential tissue-specific effects, other possible reasons for this difference may include

Fig. 4 Effect of WISP1 on insulin signalling in AML12 hepatocytes. Representative western blots and bar graphs show the effects of WISP1 on insulin-stimulated phosphorylation of Akt-Thr308 (a), Akt-Ser473 (b), GSK3 β -Ser9 (c), p70S6 kinase (K)-Thr389 (d), IR β -Tyr1150/1151 (e) and FOXO1-Ser256 (f) and on protein abundance of IRS1 (g). The dividing lines in the blots in (g) indicate places where the blot has been cut. The scattered bar graphs indicate the mean \pm SD for the phosphorylation levels obtained in three or four independent experiments. The phosphorylation levels were normalised for the protein abundances of the non-phosphorylated protein, GAPDH or α -tubulin, as indicated. The values obtained in cells incubated with 100 nmol/l insulin only were considered as the control and set at 100%. The effects of WISP1 and insulin were analysed by two-way ANOVA with Bonferroni correction for multiple comparisons. * p < 0.05 and ** p < 0.01, with vs without WISP1 incubation; † p < 0.05, †† p < 0.01 and ††† p < 0.001, with vs without insulin stimulation



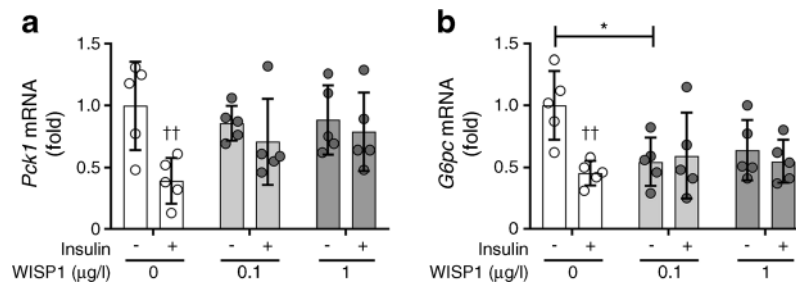


Fig. 5 WISP1 impairs the insulin-mediated suppression of gluconeogenic gene expression in primary hepatocytes. Hepatocytes were exposed to WISP1 for 24 h. The last 60 min of the WISP1 incubation was carried out in the absence or presence of insulin (100 nmol/l). *Pck1* (a) and *G6pc* (b) expression levels were assessed by quantitative RT-PCR and normalised to 18S. Data represent mean ± SD of the

expression levels of five independent experiments using cells from different mice. The values obtained for untreated cells were considered as controls and set as 1. Differences among conditions were calculated by two-way ANOVA and Bonferroni correction for multiple comparisons. * $p < 0.05$, with vs without WISP1 incubation; †† $p < 0.01$, with vs without insulin stimulation

the duration of WISP1 exposure (24 h in the present study vs 30 min in the 3T3-L1 experiment) as well as the concentration of WISP1 applied. Here, we used WISP1 concentrations representative of the levels found in the circulation whereas the 3T3-L1 adipocytes were exposed to supraphysiological concentrations of WISP1. Moreover, different signalling pathways might be involved in the actions of WISP1 in various cell types [27]. Importantly, the impaired insulin-mediated Akt phosphorylation in primary hSkMCs and AML12 hepatocytes

was accompanied by inhibition of the phosphorylation of several of its well-characterised substrates. In myotubes, reductions in the insulin-mediated phosphorylation of GSK3β and p70S6K paralleled the inhibition of Akt phosphorylation. Strikingly, in AML12 hepatocytes, the insulin-mediated phosphorylation of GSK3β was not impaired by recombinant WISP1, whereas the phosphorylation of p70S6K and FOXO1 were markedly reduced or even abrogated. Tissue-specific differences in the phosphorylation of Akt substrates

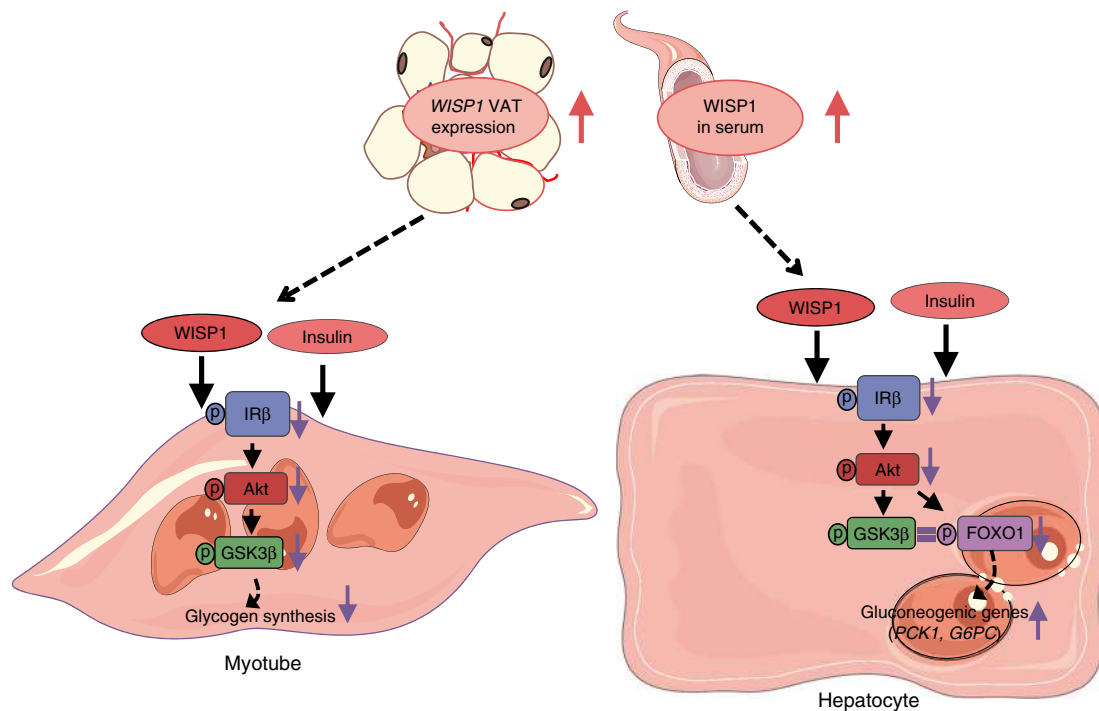


Fig. 6 Schematic diagram showing how WISP1 impairs insulin action in myotubes and hepatocytes. During obesity, *WISP1* VAT expression and WISP1 serum levels increase. In vitro, physiological concentrations of WISP1 impair insulin action on phosphorylation of IRβ, Akt and GSK3β in myotubes and reduce insulin-mediated phosphorylation of IRβ, Akt, GSK3β and FOXO1 in hepatocytes (purple arrow downwards). WISP1 abrogates insulin-mediated induction of glycogen synthesis in myotubes

(purple arrow downwards) and insulin-induced suppression of gluconeogenic gene expression (purple arrow upwards). Examined effects (solid arrow), potential effects (dotted arrow). Images ©Les Laboratoires Servier, courtesy of Servier Medical Art (<https://smart.servier.com/>), reproduced under the Creative Commons Attribution 3.0 France (CC BY 3.0 FR) license (<https://creativecommons.org/licenses/by/3.0/fr/>)

have also been reported by others [29, 30]. Gene silencing experiments in cultured cells as well as studies on mouse models suggest that tissue-specific changes in the abundance of Akt isoforms as well as the insulin receptor substrates IRS1 and IRS2 contribute to Akt substrate selection [29, 30].

As previously mentioned, WISP1 is a proinflammatory adipokine [13] and circulating WISP1 showed an association with systemic levels of IL-8 [15]. In our study, we further reveal the association between circulating WISP1 and markers of adipose tissue and systemic inflammation. Interestingly, the serum HO-1 level was the strongest predictor of circulating WISP1, even after adjustment for age and BMI. HO-1, encoded by *HMOX1*, is a stress-induced protein that is critical for stem cell differentiation [31]. Induction of the *Hmox1* gene in mice is protective against deleterious obesity phenotype as characterised by a reduced number of enlarged adipocytes, an increased number of small adipocytes and a higher adiponectin concentration [24]. Moreover, increased HO-1 expression in human mesenchymal stem cell-derived adipocytes decreases differentiation and lipid accumulation of adipocytes via upregulation of the Wnt signalling cascade [23]. These data allow us to speculate that upregulation of Wnt signalling by HO-1 leads to higher expression levels and possibly production of WISP1 from younger adipocytes. This, in turn, might result in the redistribution of in-tissue and in-body insulin sensitivity and an increased insulin resistance in older cells (i.e. redistribution of glucose and other substrates with better supply of young and proliferating cells).

A limitation of the present study is that we could not detail the mechanism by which WISP1 inhibits insulin action especially because of the high complexity of the Wnt signalling pathway [3]. Based on the modular structure of WISP1, several functional receptors have been identified. Previous studies showed that WISP1 interacted with integrin $\alpha(5)\beta$ in human bone marrow stromal cells [32]. Furthermore, WISP1 has been shown to bind decorin and biglycan in the extracellular matrix of fibroblasts [33]. The presence of an IGF binding protein domain in CCN proteins including WISP1 suggests an interference with the IGF and insulin signalling pathway [34–36]. Our observations showed a decrease in the insulin-mediated tyrosine phosphorylation of insulin receptor- β /IGF-1 receptor by WISP1, indicating that WISP1 might act by interfering with these receptors in myotubes and hepatocytes. The inhibition of insulin action by adipo(cyto)kines is frequently associated with a decreased protein abundance of IRS1 [19, 25]. Although WISP1 was found to decrease the abundance of IRS1 in myotubes, this was not the case in AML12 hepatocytes. The latter cell line even displayed an increase in IRS1 protein levels following WISP1 exposure, whereas the abundance of IRS2, which is considered to be the major insulin receptor substrate in the liver [25], was unaltered by WISP1 treatment. This raises the possibility that WISP1 may use alternative mechanisms to impair insulin action in myotubes and hepatocytes.

The second limitation of our study is that it was conducted in men (but not in women), and that the OGTT data were collected in a subgroup of the obese men. Without additional studies, caution should be taken when generalising these findings to the entire population.

Taken together, our study identifies WISP1 as a determinant of obesity in men. The increases in circulating WISP1 levels associate with variables reflecting insulin resistance in vivo in individuals with obesity. In vitro studies corroborate the associations observed in the clinical study by showing that recombinant WISP1 impairs insulin signalling in myotubes and hepatocytes with consequent impaired gluconeogenesis and glycogen synthesis (Fig. 6). Thus, WISP1 may regulate whole-body insulin sensitivity and glucose uptake due to its effects on insulin signalling in liver and muscle and is an attractive target for the prevention and treatment of obesity and diabetes.

Acknowledgements Part of this work was presented as an abstract at the 52nd European Association for the Study of Diabetes (EASD) Annual Meeting 2016.

Data availability The datasets analysed in this study are available from the corresponding author upon reasonable request.

Funding This work was financially supported by a grant to NR and DMO from the German Center for Diabetes Research ('WISP1 is a novel target for regulation of glucose metabolism'), by a grant to NR and DMO from European Foundation for Study of Diabetes (EFS/AZ Cellular Plasticity, 'Unravelling the role of WISP1 on metabolic and cellular plasticity in white adipose tissue') and by an internal grant of the German Institute of Human Nutrition to MM (2017, 'Effects of dietary protein intake on lipid metabolism and inflammatory markers in human adipose tissue').

Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

Contribution statement TH, NR, OP and DMO designed the study, conducted experiments, performed data analysis and drafted the manuscript. CT, MM, WJ, AFHP, SH, AR, VL, GHT and AS conducted experiments, performed data analysis and drafted the manuscript. DHdW, MR, NS and EF conducted experiments, performed data analysis and reviewed the manuscript. FVdV and MB collected clinical samples, analysed clinical data, maintained participants records, supervised clinical chemistry and reviewed the manuscript. YVN and BL designed and supervised the clinical study and reviewed the manuscript. OK performed statistical analysis and reviewed the manuscript. DMO and OP are the guarantors of this work, had full access to all the data and take full responsibility for the integrity of the data and the accuracy of the data analysis. All authors have seen and approved the final version of the manuscript.

References

1. Gustafson B, Hammarstedt A, Hedjazifar S, Smith U (2013) Restricted adipogenesis in hypertrophic obesity: the role of WISP2, WNT, and BMP4. *Diabetes* 62:2997–3004
2. Gustafson B, Hedjazifar S, Gogg S, Hammarstedt A, Smith U (2015) Insulin resistance and impaired adipogenesis. *Trends Endocrinol Metab* 26:193–200

3. Niehrs C (2012) The complex world of WNT receptor signalling. *Nat Rev Mol Cell Biol* 13:767–779
4. Cruciat CM, Niehrs C (2013) Secreted and transmembrane wnt inhibitors and activators. *Cold Spring Harb Perspect Biol* 5:a015081
5. Clevers H, Nusse R (2012) Wnt/ β -catenin signaling and disease. *Cell* 149:1192–1205
6. Nusse R, Clevers H (2017) Wnt/ β -catenin signaling, disease, and emerging therapeutic modalities. *Cell* 169:985–999
7. Zeng X, Huang H, Tamai K et al (2008) Initiation of Wnt signaling: control of Wnt coreceptor Lrp6 phosphorylation/activation via frizzled, dishevelled and axin functions. *Development* 135:367–375
8. Zeng X, Tamai K, Doble B et al (2005) A dual-kinase mechanism for Wnt co-receptor phosphorylation and activation. *Nature* 438:873–877
9. Palsgaard J, Emanuelli B, Winnay JN, Sumara G, Karsenty G, Kahn CR (2012) Cross-talk between insulin and Wnt signaling in preadipocytes: role of Wnt co-receptor low density lipoprotein receptor-related protein-5 (LRP5). *J Biol Chem* 287:12016–12026
10. Dahlman I, Elsen M, Tennagels N et al (2012) Functional annotation of the human fat cell secretome. *Arch Physiol Biochem* 118:84–91
11. Ehlund A, Mejhert N, Lorente-Cebrian S et al (2013) Characterization of the Wnt inhibitors secreted frizzled-related proteins (SFRPs) in human adipose tissue. *J Clin Endocrinol Metab* 98:E503–E508
12. Ouchi N, Higuchi A, Ohashi K et al (2010) Sfrp5 is an anti-inflammatory adipokine that modulates metabolic dysfunction in obesity. *Science* 329:454–457
13. Murahovschi V, Pivovarova O, Ilkavets I et al (2015) WISP1 is a novel adipokine linked to inflammation in obesity. *Diabetes* 64:856–866
14. Sahin Ersoy G, Altun Ensari T, Subas S, Giray B, Simsek EE, Cevik O (2016) WISP1 is a novel adipokine linked to metabolic parameters in gestational diabetes mellitus. *J Matern Fetal Neonatal Med* 1–5
15. Barchetta I, Cimini FA, Capoccia D et al (2017) WISP1 is a marker of systemic and adipose tissue inflammation in dysmetabolic subjects with or without type 2 diabetes. *J Endocr Soc* 1:660–670
16. Diabetes AATFoI (2006) American College of Endocrinology and American Diabetes Association Consensus statement on inpatient diabetes and glycemic control. *Diabetes Care* 29:1955–1962
17. Ruige JB, Bekaert M, Lapauw B et al (2012) Sex steroid-induced changes in circulating monocyte chemoattractant protein-1 levels may contribute to metabolic dysfunction in obese men. *J Clin Endocrinol Metab* 97:E1187–E1191
18. Bekaert M, Ouwens DM, Horbelt T et al (2016) Reduced expression of chemerin in visceral adipose tissue associates with hepatic steatosis in patients with obesity. *Obesity (Silver Spring)* 24:2544–2552
19. Wiza C, Herzfeld de Wiza D, Nascimento EB, Lehr S, Al-Hasani H, Ouwens DM (2013) Knockdown of PRAS40 inhibits insulin action via proteasome-mediated degradation of IRS1 in primary human skeletal muscle cells. *Diabetologia* 56:1118–1128
20. Jelenik T, Kaul K, Seuaris G et al (2017) Mechanisms of insulin resistance in primary and secondary nonalcoholic fatty liver. *Diabetes* 66:2241–2253
21. Akie TE, Cooper MP (2015) Determination of fatty acid oxidation and lipogenesis in mouse primary hepatocytes. *J Vis Exp* e52982
22. Gutt M, Davis CL, Spitzer SB et al (2000) Validation of the insulin sensitivity index (ISI_{0,120}): comparison with other measures. *Diabetes Res Clin Pract* 47:177–184
23. Vanella L, Sodhi K, Kim DH et al (2013) Increased heme-oxygenase 1 expression in mesenchymal stem cell-derived adipocytes decreases differentiation and lipid accumulation via upregulation of the canonical Wnt signaling cascade. *Stem Cell Res Ther* 4:28
24. Li M, Kim DH, Tsenovoy PL et al (2008) Treatment of obese diabetic mice with a heme oxygenase inducer reduces visceral and subcutaneous adiposity, increases adiponectin levels, and improves insulin sensitivity and glucose tolerance. *Diabetes* 57:1526–1535
25. Eckstein SS, Weigert C, Lehmann R (2017) Divergent roles of IRS (insulin receptor substrate) 1 and 2 in liver and skeletal muscle. *Curr Med Chem* 24:1827–1852
26. Tacke C, Aleksandrova K, Rehfeldt M et al (2017) Assessment of circulating Wnt1 inducible signalling pathway protein 1 (WISP-1)/CCN4 as a novel biomarker of obesity. *J Cell Commun Signal*. <https://doi.org/10.1007/s12079-017-0427-1>
27. Ferrand N, Bereziat V, Moldes M, Zaoui M, Larsen AK, Sabbah M (2017) WISP1/CCN4 inhibits adipocyte differentiation through repression of PPAR γ activity. *Sci Rep* 7:1749
28. Maeda A, Ono M, Holmbeck K et al (2015) WNT1-induced secreted protein-1 (WISP1), a novel regulator of bone turnover and WNT signaling. *J Biol Chem* 290:14004–14018
29. Gonzalez E, McGraw TE (2009) The Akt kinases: isoform specificity in metabolism and cancer. *Cell Cycle (Georgetown, Tex)* 8:2502–2508
30. Bouzakri K, Zachrisson A, Al-Khalili L et al (2006) siRNA-based gene silencing reveals specialized roles of IRS-1/Akt2 and IRS-2/Akt1 in glucose and lipid metabolism in human skeletal muscle. *Cell Metab* 4:89–96
31. Kozakowska M, Szade K, Dulak J, Jozkowicz A (2014) Role of heme oxygenase-1 in postnatal differentiation of stem cells: a possible cross-talk with microRNAs. *Antioxid Redox Signal* 20:1827–1850
32. Ono M, Inkson CA, Kilts TM, Young MF (2011) WISP-1/CCN4 regulates osteogenesis by enhancing BMP-2 activity. *J Bone Miner Res* 26:193–208
33. Desnoyers L, Arnott D, Pennica D (2001) WISP-1 binds to decorin and biglycan. *J Biol Chem* 276:47599–47607
34. Lorenzatti G, Huang W, Pal A, Cabanillas AM, Kleer CG (2011) CCN6 (WISP3) decreases ZEB1-mediated EMT and invasion by attenuation of IGF-1 receptor signaling in breast cancer. *J Cell Sci* 124:1752–1758
35. Lopez-Bermejo A, Khosravi J, Fernandez-Real JM et al (2006) Insulin resistance is associated with increased serum concentration of IGF-binding protein-related protein 1 (IGFBP-rP1/MAC25). *Diabetes* 55:2333–2339
36. Yamanaka Y, Wilson EM, Rosenfeld RG, Oh Y (1997) Inhibition of insulin receptor activation by insulin-like growth factor binding proteins. *J Biol Chem* 272:30729–30734