

University of Warwick institutional repository: <http://go.warwick.ac.uk/wrap>

This paper is made available online in accordance with publisher policies. Please scroll down to view the document itself. Please refer to the repository record for this item and our policy information available from the repository home page for further information.

To see the final version of this paper please visit the publisher's website. Access to the published version may require a subscription.

Author(s): Christian von Loeffelholz, Matthias Möhlig, Ayman M Arafat, Frank Isken, Joachim Spranger, Knut Mai, Harpal S Randeva, Andreas F H Pfeiffer and Martin O Weickert

Article Title: Circulating vaspin is unrelated to insulin sensitivity in a cohort of nondiabetic humans

Year of publication: 2010

Link to published article:

<http://dx.doi.org/10.1530/EJE-09-0737>

Publisher statement: Disclaimer. This is not the definitive version of record of this article. This manuscript has been accepted for publication in European Journal of Endocrinology, but the version presented here has not yet been copy edited, formatted or proofed. Consequently, the journal accepts no responsibility for any errors or omissions it may contain. The definitive version is now freely available at <http://dx.doi.org/10.1530/EJE-09-0737>. © 2010 European Society of Endocrinology

Circulating vaspin is unrelated to insulin sensitivity in a cohort of non-diabetic humans

Short running title: serum vaspin and insulin sensitivity

Christian von Loeffelholz¹, Matthias Möhlig¹, Ayman M. Arafat¹, Frank Isken¹, Joachim Spranger¹, Knut Mai¹, Harpal S. Randeva², Andreas F. H. Pfeiffer¹, Martin O. Weickert^{1,2}

¹Department of Clinical Nutrition, German Institute of Human Nutrition Potsdam-Rehbruecke, 14558 Nuthetal, Germany; and Department of Endocrinology, Diabetes and Nutrition, Charité'-University-Medicine, Campus Benjamin Franklin, 12200 Berlin, Germany

²Warwickshire Institute for the Study of Diabetes, Endocrinology and Metabolism, University Hospitals Coventry and Warwickshire NHS Trust, CV2 2DX, Coventry, UK, and Clinical Sciences Research Institute, Warwick Medical School, University of Warwick, CV4 7AL, Coventry, UK

Correspondence:

Martin O. Weickert, MD

Warwickshire Institute for the Study of Diabetes, Endocrinology and Metabolism

University Hospitals Coventry and Warwickshire

CV2 2DX

Coventry, UK

Tel: 0044 2476 965972; Fax: 00442476 965964

Email: m.weickert@warwick.ac.uk

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; FFA, free fatty acids; GIR, glucose infusion rate; HDL, high density lipoprotein; HOMA-IR, Homeostasis-model-assessment for insulin resistance; IGT, impaired glucose tolerance; LDL, low density lipoprotein; NGM, normal glucose metabolism; OC, oral contraceptives; OGTT, oral glucose tolerance test; PPI, proton pump inhibitors; SBP, systolic blood pressure; WAT, white adipose tissue

Abstract

Objective: To study the association of vaspin with glucose metabolism.

Design: Cross-sectional and intervention study.

Subjects and methods: The association of serum vaspin with metabolic and anthropometric characteristics was investigated in 108 volunteers. Euglycemic-hyperinsulinemic clamps (EHC) were performed in 83 of the participants. Changes of circulating vaspin levels were additionally studied in a cross-over study using 300-min EHC with lipid vs saline infusion (n = 10).

Results: Neither glucose tolerance status nor insulin sensitivity, both as measured using EHCs and using homeostasis-model-assessment for insulin resistance (HOMA-IR), were significantly associated with serum vaspin in the cross-sectional study. Further, there was no effect of short-term lipid induced insulin resistance due to a 300 min intravenous lipid challenge on circulating vaspin. However, circulating vaspin levels were significantly elevated in women using oral contraceptives (OC), both compared to women without OC intake (1.17 ± 0.26 vs 0.52 ± 0.09 ng/ml, $P = 0.02$) and males (1.17 ± 0.26 vs 0.29 ± 0.04 ng/ml, $P = 0.01$). After exclusion of OC using females and stratification according to body mass index (BMI), a significant sexual dimorphism in subjects with a BMI < 25 kg/m² was observed (males 0.21 ± 0.04 ng/ml vs females 0.70 ± 0.16 ng/ml, $P = 0.009$).

Conclusion: Our results support the existence of a sexual dimorphism regarding circulating vaspin. The lack of an association of serum vaspin with HOMA-IR and M-value indicates, however, no major role for vaspin concerning insulin sensitivity in non-diabetic humans.

Introduction

Adipose tissue, recognized today as an active secretory organ, modifies the metabolic state of insulin-dependent tissues (1). The protease inhibitor vaspin is a member of the superfamily of serpins and was isolated from white adipose tissue (WAT) of Otsuka Long-Evans Tokushima fatty (OLETF) rats (2) and humans (3). Observations in animals suggest vaspin to exert insulin sensitizing effects mainly targeted on WAT, and diabetic treatment to increase mRNA expression and serum levels of vaspin (4). Therefore it has been proposed that the up-regulation of vaspin represents a compensatory mechanism against rising insulin resistance (4,5). Improved insulin sensitivity and altered gene expression of insulin resistance candidate genes after administration of recombinant vaspin to a mouse model of diet-induced obesity provide support for this hypothesis (2,5).

In humans with normal glucose metabolism (NGM), serum vaspin has been reported to be significantly negatively associated with the body mass index (BMI)-adjusted glucose infusion rate (GIR) during steady state of a euglycemic hyperinsulinemic clamp (EHC), and after four weeks of physical training the change in serum vaspin correlates positively with the change in GIR in a sample of 60 volunteers (6). Further, significant associations between circulating vaspin and estimates of insulin resistance such as homeostasis-model-assessment (HOMA-IR) were reported. One study supports i.e. a negative correlation between circulating vaspin and HOMA-IR (7), while the change in HOMA-IR after weight loss was positively associated with altered serum vaspin levels (8,9). Moreover, circulating vaspin shows significant associations with several anthropometric and metabolic markers (6,7,8,9,10,11). Additionally, the results of a cross-sectional study suggest the existence of a sexual dimorphism, with higher circulating vaspin levels in women *vs* men (10). Yet, this gender difference might be influenced by glycemic control (6) or further factors, given that other recent studies showed conflicting results (9,11). In concert with the fact that vaspin is mainly expressed in visceral

adipose tissue (VAT) (3), current knowledge suggests that serum levels differ depending on sex, BMI, metabolic control and fat mass.

Since in humans modulating factors leading to changes in circulating vaspin are largely unknown, we studied associations of serum vaspin with anthropometric and metabolic characteristics in a sample of 108 male and female volunteers with a wide range of BMI, age, and metabolic control. Further, we analysed the association of serum vaspin with peripheral insulin sensitivity measured by EHC in a subset of 83 volunteers. Moreover, it is unknown whether the regulation of circulating vaspin in humans is mediated by altered insulin resistance or by associated changes in glycemic control. Lipid infusion is recognized to acutely produce peripheral insulin resistance within a range of about 200 minutes (12). Thus, we further hypothesised that a short-term lipid challenge using conditions known to significantly produce peripheral insulin resistance (13) could impact serum vaspin.

Subjects and Methods

Subjects

The experimental protocol was approved by the local Ethics Committee. All participants gave written informed consent before starting the study.

Cross-sectional study: One-hundred-and-eight male and female participants were included in the cross-sectional study. In order to yield stratified groups according to BMI (lean: < 18.5 kg/m²; normal weight: 18.5-24.9 kg/m²; overweight: 25.0-29.9 kg/m²; obese: ≥ 30.0 kg/m²) and gender, volunteers were selected as a subset from the cross-sectional Metabolic Syndrome Berlin Potsdam (MeSyBePo) study. Details of recruitment were recently published (14). All volunteers were screened for serious health problems. Patients with a history of renal or hepatic dysfunction, type 2 diabetes, abnormal thyroid function, or glucocorticoid therapy

were excluded. A subset of volunteers (n = 83; 40 males; mean age 50.5 ± 1.6 years, mean BMI 28.1 ± 0.7 kg/m²) additionally underwent EHCs.

Lipid vs saline intervention study: A further subset of healthy volunteers with NGM was enrolled (n = 10; 5 males; mean age 50.6 ± 4.2 years, mean BMI 24.3 ± 1.1 kg/m²). Fertile female subjects were studied in the early follicular phase of the menstrual cycle. Any renal, vascular, or hepatic diseases were exclusion criteria, as well as a history of smoking or any diabetes or thyroid medication.

Experimental Design and Assays

Cross-sectional study: After arrival at the metabolic unit medical history was taken, and the subjects underwent physical examination. Fasting venous blood samples were collected and stored at -80°C until analysis. Glucose tolerance was tested in all of the subjects by oral glucose tolerance tests (OGTT). All the participants of this study had a fasting capillary glucose < 5.0 mmol/l and as such normal fasting glucose levels. Participants with NGM were defined as having both a normal fasting glucose and a glucose value of < 7.8 mmol/l at the 120 min OGTT value. Participants with IGT had a normal fasting glucose but a capillary glucose at the 120 min OGTT value ≥ 7.8 and < 11.1 mmol/l.

Anthropometric data, as well as arterial blood pressure according to Riva Rocci, were obtained as reported previously (14). For the estimation of fasting insulin sensitivity, HOMA-IR according to the formula $[\{\text{fasting glucose (mmol/l)} \times (\text{fasting insulin (pmol/l)}/6)\}/22.5]$ was applied. EHCs were performed as detailed previously (15, 16). In brief, at -10 min, a bolus of insulin was administered over 10 min, adjusted for the body weight of the participants, and followed by euglycemic-hyperinsulinemic clamps for at least 2 h, using $40 \text{ mU} \times \text{m}^{-2} \times \text{min}^{-1}$ human insulin (Novo Nordisk, Bagsvaard, Denmark) and a variable infusion of glucose 20% (Serag Wiessner, Naila, Germany). Arterialized plasma glucose was adjusted at 4.4 mmol/l throughout the clamps. Whole-body glucose disposal [expressed as

insulin-mediated glucose uptake (M-value)] was calculated from the glucose infusion rate, which was constant during the last 30 min of the respective clamps.

Lipid vs saline intervention study: Participants of the lipid vs saline cross-over intervention study first underwent EHC until steady state conditions were obtained. Thereafter, EHCs were extended for a further 300 min, as detailed recently (16). A constant infusion of a lipid-solution (Deltalipid LCT 20%; Deltaselect, Pfullingen, Germany; $1.25 \text{ ml} \times \text{min}^{-1}$; contents in 1.000 ml: soybean oil 200 g, glycerol 25.9 g, egg phospholipids 12 g, oleate 0.3 g; supplemented with Heparin-Natrium-25000-ratiopharm; Merckle, Blaubeuren, Germany; $0.4 \text{ IU} \times \text{kg}^{-1} \times \text{min}^{-1}$) was added to raise free fatty acid (FFA) concentrations. All participants returned to the metabolic unit on a separate day for the control experiments (washout 279 ± 15 days), which followed the same protocol, apart that saline (saline 0.9%; Fresenius Kabi, Bad Homburg, Germany; $1.25 \text{ ml} \times \text{min}^{-1}$) was infused instead of lipid-heparin solution. Blood samples were drawn at timed intervals, immediately chilled and centrifuged, and the supernatants were stored at -80°C until analysis. Arterialized plasma glucose concentrations were measured immediately using the glucose oxidase method on a Dr. Müller Super-GL glucose analyzer (Freital, Germany). For the measurements of serum vaspin, blood was collected in serum tubes (Bayer, Leverkusen, Germany).

Assays

Serum vaspin was measured using a commercially available ELISA (Adipogen, Seoul, South Korea; intra-assay CV 0.6-2.8%). A relevant interference of lipidemic plasma with the vaspin assay was excluded by prior *in vitro* experiments (data not shown). Measurements of insulin in serum and FFA in plasma were performed with Cobas Mira (Roche, Lörrach, Germany). HbA1c was measured using HA 8140 (Menarini Diagnostics, Berlin, Germany). HDL and

total cholesterol were detected with ABX Pentra 400 (ABX Diagnostics, Montpellier, France), while LDL was calculated according to the Friedewald's formula.

Statistical analysis and calculations

SPSS 16.0 (Chicago, IL) was used for statistical analysis. If not stated otherwise, data are shown as means \pm SE. Presence of normal distribution was analysed by the Kolmogorov-Smirnov test. Not-normally distributed data (*i.e.* HOMA-IR) were logarithmically transformed. In the cross-sectional study, two-way ANOVA was applied to detect combined effects (*i.e.* sex \times BMI as a categorical variable) on serum vaspin. Student's t-test for independent samples was used for subgroup analyses if data were normally distributed. Otherwise Mann-Whitney U test was applied. The gender distribution within the BMI quartiles was compared by a Chi-Square test. Changes of circulating vaspin during lipid vs saline clamps were analysed using repeated-measures ANOVA with treatment and time as within-subject factors and Huynh-Feldt epsilon correction. Subgroup analyses were performed using two-tailed Student's t-test for paired samples. Linear relationships were tested by least square regression analysis. Significance level was defined as $P < 0.05$.

Results

Cross-sectional study

From the 120 initially identified subjects 12 had to be excluded because they did not meet all of the inclusion criteria. Baseline characteristics of the 108 study subjects are summarized in Table 1. The gender distribution within the BMI quartiles was not different ($P = 0.75$). The study population showed a wide variety of age (range: 21.1-72.4 years) and BMI (range: 15.8-47.8 kg/m²). Regarding the 83 individuals with available EHC data, M-value was 4.70 ± 0.33

and $4.33 \pm 0.32 \text{ mg} \times \text{kg}^{-1} \times \text{min}^{-1}$ for males and females, respectively, with no significant difference between genders ($P = 0.40$).

When analysing the entire study group serum vaspin ranged from 0.05 to 3.45 ng/ml, with a mean of 0.50 ± 0.06 ng/ml. Surprisingly, we found the highest serum vaspin levels in normal-weight subjects (lean: 0.58 ± 0.18 ng/ml; normal weight: 0.76 ± 0.14 ng/ml; overweight: 0.29 ± 0.81 ng/ml; obese: 0.34 ± 0.05 ng/ml). Further, we observed significantly higher serum vaspin levels in women vs men (0.66 ± 0.10 vs 0.29 ± 0.04 ng/ml, $P = 0.001$). However, regression analysis indicated that the variance of serum vaspin within the entire group of females was significantly associated with the usage of oral contraceptives (OC) ($B = 0.282$, $P = 0.03$; $R^2 = 0.30$, $P < 0.001$ for the total model), and this effect remained statistically significant after excluding subjects with IGT (OC: $B = 0.325$, $P = 0.03$; $R^2 = 0.365$, $P = 0.001$ for the total model). Additionally, females using OC showed significantly higher serum vaspin levels vs females without OC usage (1.17 ± 0.26 vs 0.52 ± 0.09 ng/ml, $P = 0.02$); Figure 1A), and males (1.17 ± 0.26 vs 0.29 ± 0.04 ng/ml, $P = 0.01$; Figure 1A).

The main outcome measure of this study was to investigate the potential associations of serum vaspin with insulin sensitivity. When analysing all participants using a model with log HOMA-IR as the dependent variable and sex, age, BMI, and glucose metabolism (NGM vs IGT) as independent variables, a significant association with serum vaspin ($B = 0.218$, $P = 0.009$) became apparent ($R^2 = 0.452$, $P < 0.001$ for the total model). However, after exclusion of OC users ($n = 13$), log HOMA-IR was significantly associated only with BMI ($B = 0.649$, $P < 0.001$), while we found no correlation with circulating vaspin ($B = 0.135$, $P = 0.10$; $R^2 = 0.416$, $P < 0.001$ for the total model; Table 2, model 1). Additionally, linear regression testing in the subgroup of 83 subjects investigated with EHC did not indicate associations of circulating vaspin ($B = -0.023$, $P = 0.83$) with insulin sensitivity expressed as log M-value, whereas, again, a strong correlation with BMI was found ($B = -0.479$, $P < 0.001$; $R^2 = 0.174$, $P = 0.001$ for the total model; Table 2, model 2).

The residual study group ($n = 95$) was stratified according to BMI quartiles. Males revealed increasing serum vaspin with BMI (lean: 0.06 ± 0.01 ng/ml; normal weight: 0.24 ± 0.05 ng/ml; overweight: 0.34 ± 0.11 ng/ml; obese: 0.31 ± 0.05 ng/ml), while in females peak concentrations were observed in normal weight subjects (lean: 0.31 ± 0.11 ng/ml; normal weight: 0.86 ± 0.21 ng/ml; overweight: 0.22 ± 0.09 ng/ml; obese: 0.37 ± 0.09 ng/ml). The gender difference concerning circulating vaspin was abrogated ($P = 0.07$; Figure 1A). However, a linear regression model adjusted for BMI ($B = -0.097$, $P = 0.34$) and age ($B = -0.143$, $P = 0.16$) revealed a significant, although small effect of sex ($B = -0.218$, $P = 0.03$; $R^2 = 0.052$, $P = 0.04$ for the total model), which persisted when IGT individuals were excluded (sex: $B = -0.304$, $P = 0.01$; $R^2 = 0.124$, $P = 0.009$ for the total model). We further divided the residual cross-sectional subjects into groups with BMI < 25 kg/m² vs BMI ≥ 25 kg/m². A significant sexual dimorphism was found in individuals with a BMI < 25 kg/m² ($P = 0.009$; Figure 1B), while no differences became apparent in overweight and obese subjects ($P = 0.91$; Figure 1B). According to this stratification, two-way ANOVA exposed a significant effect of sex ($P = 0.04$) and sex \times BMI ($P = 0.03$) on circulating vaspin levels. Thus, our results support the hypothesis of a gender effect on circulating vaspin in lean and normal weight subjects, while the usage of OC could exert further relevant effects on the regulation of serum levels.

When dividing the residual group according to IGT vs NGM (Figure 1C), two-way ANOVA exposed no effect of IGT ($P = 0.59$) or sex \times IGT ($P = 0.14$) on serum vaspin. Also after stratification according to BMI, there was neither a significant impact of IGT ($P = 0.60$ and $P = 0.09$ for BMI < 25 kg/m² vs BMI ≥ 25 kg/m², respectively), nor did we find a significant interaction of sex and IGT ($P = 0.91$ and $P = 0.80$ for BMI < 25 kg/m² vs BMI ≥ 25 kg/m², respectively).

Lipid vs saline intervention study

We further investigated whether induction of insulin resistance via lipid infusion affects circulating vaspin. During both, saline and lipid intervention, plasma glucose levels were clamped and thus remained stable at a level of 4.6 ± 0.1 mmol/l without significant differences between groups (treatment \times group effect: $P = 0.13$; Figure 2A). Steady state was reached at a mean of 137.6 ± 9.0 min.

As expected following insulin infusion, a significant time \times treatment effect concerning serum insulin was observed (baseline vs all other time points: $P < 0.001$ for both, lipid and saline infusion, respectively), which was comparable between groups (treatment \times group effect: $P = 0.43$; Figure 2B). Plasma FFA initially decreased according to insulin infusion during both, lipid and saline intervention (time \times treatment effect: $P < 0.001$, respectively; Figure 2C). However, plasma FFA over time rose significantly only during the lipid clamps (time \times treatment effect: $P = 0.02$; Figure 2C), and the increment in plasma FFA was significant in comparison to saline intervention (treatment \times group effect: $P = 0.001$; Figure 2C).

As expected from our experimental design, peripheral insulin sensitivity significantly decreased in lipid-exposed subjects (baseline M-value 6.98 ± 0.76 vs 5.24 ± 0.91 $\text{mg} \times \text{kg}^{-1} \times \text{min}^{-1}$ after lipid challenge; $P = 0.008$) but not during the saline clamps (baseline M-value 6.55 ± 0.86 vs 6.75 ± 0.59 $\text{mg} \times \text{kg}^{-1} \times \text{min}^{-1}$ after saline; $P = 0.67$). Despite euglycemic hyperinsulinemia and insulin resistance with significantly raised plasma free fatty acids during lipid intervention, neither a time \times treatment effect ($P = 0.21$, $P = 0.37$ for saline and lipid infusion, respectively) nor a significant difference between lipid and saline EHCs was observed concerning serum vaspin levels (treatment \times group effect: $P = 0.44$; Figure 2D).

Discussion

Previous studies suggest a significant association of serum vaspin with insulin resistance, both as estimated using HOMA-IR (7,8,9) and when measured using EHCs as the gold standard method for the measurement of insulin sensitivity (6). However, although we confirmed an association of circulating vaspin with HOMA-IR in the entire study group, this effect was abolished after excluding OC users, indicating a strong influence of OC usage on circulating vaspin. Consequently, OC users were excluded from further analyses. No association of circulating vaspin with M-value derived from EHCs was found, and also lipid-induced insulin resistance did not affect serum vaspin.

In contrast to our findings, a recent investigation reports serum vaspin to correlate negatively with the BMI-adjusted GIR in NGM humans, while no association was shown in type 2 diabetic patients (6). These differences in findings could be partly explained by the varying characteristics of the here investigated subjects compared to the participants in the mentioned study. More importantly, no adjustment for OC usage was reported in the mentioned study, which might have contributed to diverse findings.

As an additional possibility, the regulation of serum vaspin might have been influenced by metabolic control, since a recent study shows a glucose dependent up-regulation of vaspin net production and secretion in human omental adipose tissue explants (8). Furthermore, in humans a positive association with glycemic control and anti-diabetic treatment was observed (7,10). However, within the present study we found neither an association of serum vaspin with HbA1c nor we detected a significant impact of IGT on circulating vaspin.

We found significantly higher circulating vaspin levels in females *vs* males, which is in accordance with some (6,10), but not all previous studies (9,11). The here observed association of circulating vaspin with OC usage could provide a potential explanation concerning the observed gender differences. This hypothesis is supported by recent data showing that treatment of omental adipose tissue from women with polycystic ovary

syndrome with different doses of 17 β -estradiol exhibits a dose-dependent, albeit insignificant, increase in vaspin levels (8). Furthermore, a significant decrease of 17 β -estradiol in response to metformin therapy, accompanied by a fall in serum vaspin, was reported (8). These findings underline the theory that OC might have influenced the variance of serum vaspin in our females. Otherwise, it is noteworthy that after exclusion of the OC users we detected an influence of gender *per se*.

The present study further supports the existence of an interaction between serum vaspin, sex and BMI, at least in normal weight and lean subjects, while no such association was detected in individuals with a BMI ≥ 25 kg/m². Since vaspin is mainly produced by VAT in humans (3), we would have expected increasing serum levels with a rising waist circumference and BMI. However, circulating vaspin increased with BMI only in males, while in the group of females, we paradoxically observed highest serum levels in normal weight subjects. In accordance with our findings, a previous study also reported significantly increased serum vaspin levels with a rising BMI in males, while in females vaspin peaked in the overweight and tended to decrease again in the obese participants (6). This U-shaped relationship between body mass and serum vaspin has been recently affirmed in elderly overweight subjects with carotid stenosis (11). Therefore, apart from further assessing the potential impact of OC usage and sex steroid hormones, investigating potential associations of circulating vaspin with body fat distribution could be of interest in future studies.

Limitations of the present study need to be addressed. We may have missed small differences between groups both in the cross-sectional study and in the lipid-saline sub-study, due to the relatively low number of participants investigated. In addition, a longer duration of the intervention may have further elucidated potentially present effects, although the lack of an association of circulating vaspin with insulin resistance in the cross-sectional study suggests the absence of a major interference.

In conclusion, we found no major association of serum vaspin with insulin sensitivity, at least in the here investigated cohort of non-diabetic humans with widely varying BMI. Our results further support the hypothesis of a sexual dimorphism concerning serum vaspin, but they also propose that interfering factors including the usage of OC should be regarded as potential confounders in future studies.

Acknowledgements

We thank A Wagner, A Ziegenhorn, K Sprengel and J Lin for excellent technical assistance. The study was financed by departmental research funds allocated to the German Institute of Human Nutrition. There are no sources of external funding to declare. All authors assured that there were no conflicts of interest.

References

1. Rabe K, Lehrke M, Parhofer KG & Broedl UC: Adipokines and insulin resistance. *Molecular Medicine* 2008 **14** 741-751.
2. Hida K, Wada J, Eguchi J, Zhang H, Baba M, Seida A, Hashimoto I, Okada T, Yasuhara A, Nakatsuka A, Shikata K, Hourai S, Futami J, Watanabe E, Matsuki Y, Hiramatsu R, Akagi S, Makino H & Kanwar YS: Visceral adipose tissue-derived serine protease inhibitor: a unique insulin-sensitizing adipocytokine in obesity. *Proceedings of the National Academy of Science U S A* 2005 **102** 10610-10615.
3. Kloting N, Berndt J, Kralisch S, Kovacs P, Fasshauer M, Schon MR, Stumvoll M & Blüher M: Vaspin gene expression in human adipose tissue: association with obesity and type 2 diabetes. *Biochemical and Biophysical Research Communications* 2006 **339** 430-436.
4. Li Q, Chen R, Moriya J, Yamakawa J, Sumino H, Kanda T & Takahashi T: A novel adipocytokine, visceral adipose tissue-derived serine protease inhibitor (vaspin), and obesity. *Journal of International Medical Research* 2008 **36** 625-629.
5. Wada J: Vaspin: a novel serpin with insulin-sensitizing effects. *Expert Opinion on Investigational Drugs* 2008 **17** 327-333.
6. Youn BS, Kloting N, Kratzsch J, Lee N, Park JW, Song ES, Ruschke K, Oberbach A, Fasshauer M, Stumvoll M & Blüher M: Serum vaspin concentrations in human obesity and type 2 diabetes. *Diabetes* 2008 **57** 372-377.
7. Gulcelik NE, Karakaya J, Gedik A, Usman A & Gurlek A: Serum vaspin levels in type 2 diabetic women in relation to microvascular complications. *European Journal of Endocrinology* 2009 **160** 65-70.
8. Tan BK, Heutling D, Chen J, Farhatullah S, Adya R, Keay SD, Kennedy CR, Lehnert H & Randeve HS: Metformin decreases the adipokine vaspin in overweight women

- with polycystic ovary syndrome concomitant with improvement in insulin sensitivity and a decrease in insulin resistance. *Diabetes* 2008 **57** 1501-1507.
9. Handisurya A, Riedl M, Vila G, Maier C, Clodi M, Prikoszovich T, Ludvik B, Prager G, Luger A & Kautzky-Willer A: Serum Vaspin Concentrations in Relation to Insulin Sensitivity Following RYGB-Induced Weight Loss. *Obesity Surgery* 2009.
 10. Seeger J, Ziegelmeier M, Bachmann A, Lossner U, Kratzsch J, Blüher M, Stumvoll M & Fasshauer M: Serum levels of the adipokine vaspin in relation to metabolic and renal parameters. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 247-251.
 11. Aust G, Richter O, Rohm S, Kerner C, Hauss J, Kloting N, Ruschke K, Kovacs P, Youn BS & Blüher M: Vaspin serum concentrations in patients with carotid stenosis. *Atherosclerosis* 2009 **204** 262-266.
 12. Boden G, Chen X, Ruiz J, White JV & Rossetti L: Mechanisms of fatty acid-induced inhibition of glucose uptake. *Journal of Clinical Investigation* 1994 **93** 2438-2446.
 13. Weickert MO, Loeffelholz CV, Roden M, Chandramouli V, Brehm A, Nowotny P, Osterhoff MA, Isken F, Spranger J, Landau BR, Pfeiffer AF & Möhlig M: A Thr94Ala mutation in human liver fatty acid-binding protein contributes to reduced hepatic glycogenolysis and blunted elevation of plasma glucose levels in lipid-exposed subjects. *American Journal of Physiology Endocrinology and Metabolism* 2007 **293** E1078-1084.
 14. Fischer A, Fisher E, Möhlig M, Schulze M, Hoffmann K, Weickert MO, Schueler R, Osterhoff M, Pfeiffer AF, Boeing H, et al.: KCNJ11 E23K affects diabetes risk and is associated with the disposition index: results of two independent German cohorts. *Diabetes Care* 2008, **31**:87-89.

15. DeFronzo RA, Tobin JD & Andres R: Glucose clamp technique: a method for quantifying insulin secretion and resistance. *American Journal of Physiology* 1979 237 E214-223.
16. Weickert MO, Möhlig M, Spranger J, Schofl C, Loeffelholz CV, Riepl RL, Otto B & Pfeiffer AF: Effects of euglycemic hyperinsulinemia and lipid infusion on circulating cholecystokinin. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 2328-2333.

Table 1 Baseline characteristics of the cross-sectional study population.

	females	males	<i>P</i>
N	62	46	-
Age (years)	46.6 ± 2.0	50.4 ± 2.2	0.21
BMI (kg/m ²)	27.7 ± 1.2	29.3 ± 0.9	0.30
Waist circumference (cm)	87.0 ± 2.8	99.3 ± 2.3	0.001
Fasting plasma glucose (mmol/l)	4.92 ± 0.07	5.12 ± 0.07	0.08
120 min OGTT-plasma glucose (mmol/l)	7.36 ± 0.18	6.80 ± 0.24	0.04
HbA1c (%)	5.20 ± 0.06	5.23 ± 0.05	0.73
HOMA-IR	1.81 ± 0.21	1.92 ± 0.22	0.73
LDL (mmol/l)	3.7 ± 0.48	3.41 ± 0.15	0.63
HDL (mmol/l)	1.54 ± 0.05	1.39 ± 0.06	0.06
Plasma triglycerides (mmol/l)	1.12 ± 0.06	1.34 ± 0.09	0.04
Plasma FFA (mmol/l)	0.59 ± 0.03	0.53 ± 0.04	0.17
SBP (mm Hg)	117 ± 2	134 ± 3	< 0.001
DBP (mm Hg)	74 ± 1	81 ± 2	0.007
IGT (n)	21	9	-
Antihypertensive medication (n)	11	13	-
Statin (n)	1	0	-
Contraceptives (n)	13	0	-
Antidepressive medication (n)	2	2	-
Others (antiobstructive inhalator, H1-Blocker, PPI) (n)	3	2	-

Means ± SEM or absolute number of subjects under medical treatment is shown.

BMI, body mass index; DBP, diastolic blood pressure; FFA, free fatty acids; HDL, high density lipoprotein; HOMA-IR, homeostasis-model-assessment for insulin resistance; IGT, impaired glucose tolerance; LDL, low density lipoprotein; OGTT, oral glucose tolerance test; PPI, proton pump inhibitors; SBP, systolic blood pressure.

Table 2 Multivariate analysis of parameters associated with HOMA-IR and M-value in the cross-sectional study.

Independent variables	Beta coefficient	P value
Model 1: Study group with females using OC excluded		log HOMA-IR
(n = 95; R ² = 0.416, P < 0.001)		
BMI	0.649	< 0.001
Sex	0.129	0.11
Age	-0.124	0.13
Serum vaspin	0.135	0.10
Model 2: EHC subgroup with females using OC excluded		log M-value
(n = 76; R ² = 0.174, P = 0.001)		
BMI	-0.479	< 0.001
Sex	0.151	0.18
Age	-0.001	0.99
Serum vaspin	-0.023	0.84

BMI, body mass index; EHC, euglycemic hyperinsulinemic clamp; HOMA-IR, homeostasis-model-assessment for insulin resistance; OC, oral contraceptives.

Figure legends

Figure 1 Gender related distribution of vaspin serum concentrations in the cross-sectional study (n = 108). Data are means \pm SEM; ■, female subjects; □, male subjects.

A: Circulating vaspin in females using OC (n = 13; left black bar) vs females without OC usage (n = 49; right black bar, $P < 0.01$) and vs male subjects (n = 46; $P = 0.027$). Females without OC usage vs males: $P = 0.07$.

B: Vaspin serum concentrations of the study group after the exclusion of OC users stratified according to BMI and gender (n = 95). BMI $< 25 \text{ kg/m}^2$, females vs males ($P = 0.009$; left bars) and BMI $\geq 25 \text{ kg/m}^2$, females vs males ($P = 0.90$; right bars).

C: Circulating vaspin of NGM (n = 78, males n = 37; $P = 0.027$) and IGT female vs male subjects (n = 27, males n = 9; $P = 0.72$).

Figure 2 Intervention study (n = 10).

EHC with 300 min lipid vs saline infusion (n = 10). Peripheral concentrations of plasma glucose (**A**), serum insulin (**B**) and plasma free fatty acids (**C**) during intervention. Data of serum vaspin (**D**) are expressed as fold difference in lipid vs saline exposed subjects relative to the overnight fasted state.

Data are means \pm SEM for n = 10 normal glucose tolerant subjects.

(B) time \times treatment effect for insulin: Baseline vs all other time points: $P < 0.001$ for both lipid and saline infusion, respectively.

(C) time \times treatment effect for FFA due to insulin infusion, $P < 0.001$. Treatment \times group effect lipid vs saline infusion, $P = 0.001$. Single time points: * $P < 0.05$, ** $P < 0.01$.

(D) time \times treatment effect for serum vaspin, $P = 0.21$. Treatment \times group effect lipid vs saline infusion, $P = 0.44$.

Figure 1

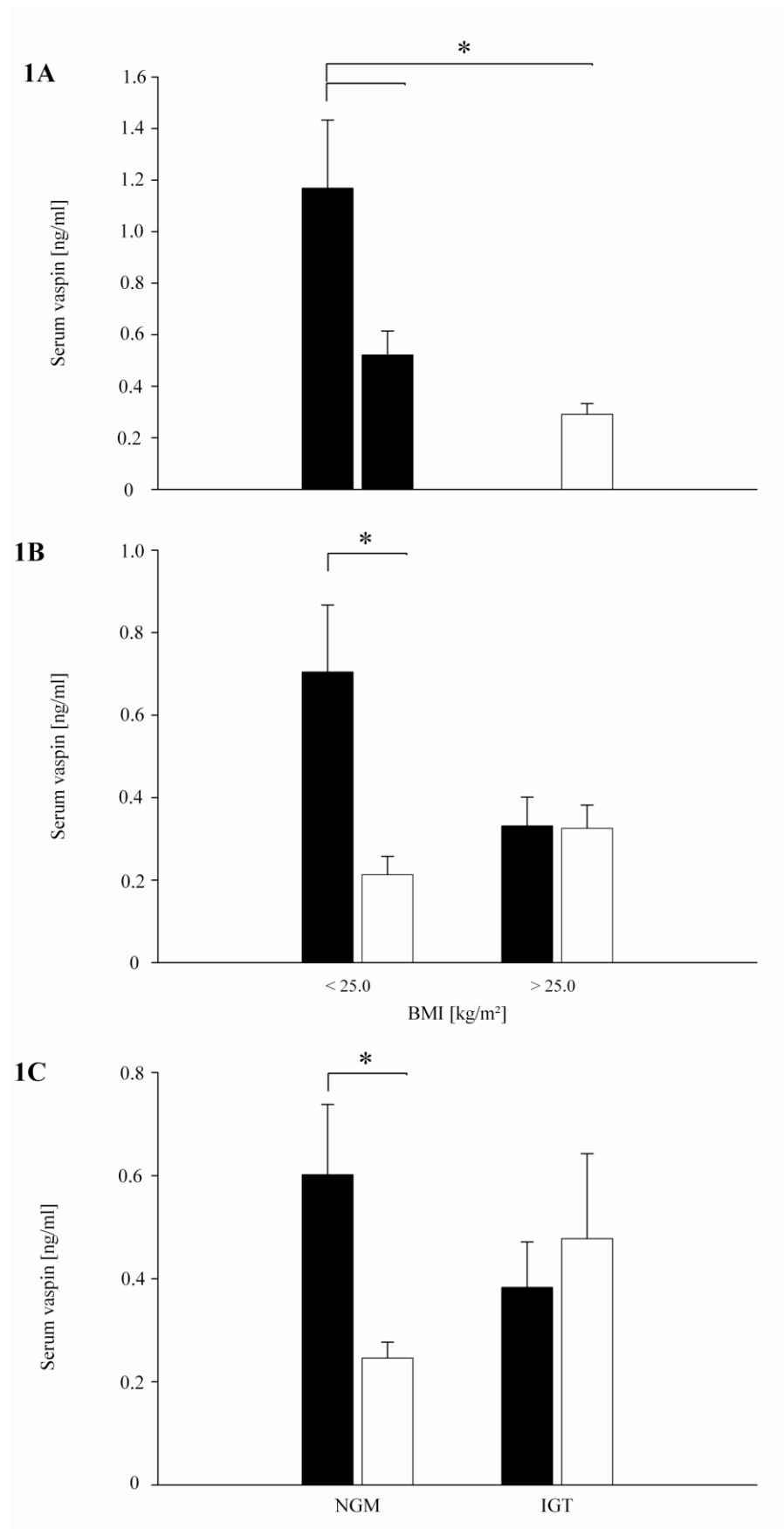


Figure 2

