

## CLINICAL STUDY

# Circulating vaspin is unrelated to insulin sensitivity in a cohort of nondiabetic humans

Christian von Loeffelholz<sup>1,2</sup>, Matthias Möhlig<sup>1,2</sup>, Ayman M Arafat<sup>1,2</sup>, Frank Isken<sup>1,2</sup>, Joachim Spranger<sup>1,2</sup>, Knut Mai<sup>1,2</sup>, Harpal S Randeva<sup>3,4</sup>, Andreas F H Pfeiffer<sup>1,2</sup> and Martin O Weickert<sup>1,2,3,4</sup>

<sup>1</sup>Department of Clinical Nutrition, German Institute of Human Nutrition Potsdam-Rehbruecke, 14558 Nuthetal, Germany, <sup>2</sup>Department of Endocrinology, Diabetes and Nutrition, Charité-University-Medicine, Campus Benjamin Franklin, 12200 Berlin, Germany, <sup>3</sup>Warwickshire Institute for the Study of Diabetes, Endocrinology and Metabolism, University Hospitals Coventry and Warwickshire NHS Trust, CV2 2DX Coventry, UK and <sup>4</sup>Warwick Medical School, Clinical Sciences Research Institute, University of Warwick, CV4 7AL Coventry, UK

(Correspondence should be addressed to M O Weickert at Warwickshire Institute for the Study of Diabetes, Endocrinology and Metabolism, University Hospitals Coventry and Warwickshire; Email: m.weickert@warwick.ac.uk)

## 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 crossover study using 300 min EHC with lipid versus 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), was significantly associated with serum vaspin in the cross-sectional study. Furthermore, 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 ( $1.17 \pm 0.26$  vs  $0.52 \pm 0.09$  ng/ml,  $P=0.02$ ) and males ( $1.17 \pm 0.26$  vs  $0.29 \pm 0.04$  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/m<sup>2</sup> was observed (males  $0.21 \pm 0.04$  ng/ml versus females  $0.70 \pm 0.16$  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 nondiabetic humans.

European Journal of Endocrinology 162 507–513

## 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 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 4 weeks of physical training, the change in serum vaspin correlates positively with the change in GIR in a sample of 60 volunteers (6). Furthermore, significant associations between circulating vaspin and estimates of insulin resistance such as homeostasis model assessment (HOMA-IR) were reported. One study supports 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 (6–11). Additionally, the results of a cross-sectional study suggest the existence of a

sexual dimorphism, with higher circulating vaspin levels in women versus 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. Furthermore, we analyzed 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 min (12). Thus, we further hypothesized 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/m<sup>2</sup>; normal weight:  $18.5$ – $24.9$  kg/m<sup>2</sup>; overweight:  $25.0$ – $29.9$  kg/m<sup>2</sup>; obese:  $\geq 30.0$  kg/m<sup>2</sup>) and gender, volunteers were selected as a subset from the cross-sectional Metabolic Syndrome Berlin Potsdam 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  $50.5 \pm 1.6$  years, mean BMI  $28.1 \pm 0.7$  kg/m<sup>2</sup>) additionally underwent EHCs.

**Lipid versus saline intervention study** A further subset of healthy volunteers with NGM was enrolled ( $n=10$ ; 5 males; mean age  $50.6 \pm 4.2$  years, mean BMI  $24.3 \pm 1.1$  kg/m<sup>2</sup>). 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 impaired glucose tolerance (IGT) had a normal fasting glucose but a capillary glucose at the 120 min OGTT value  $\geq 7.8$  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 ((fasting glucose (mmol/l)  $\times$  (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 EHCs for at least 2 h, using  $40$  mU/m<sup>2</sup> per min human insulin (Novo Nordisk, Bagsvaerd, 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 GIR, which was constant during the last 30 min of the respective clamps.

**Lipid versus saline intervention study** Participants of the lipid versus saline crossover 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;  $1.25$  ml/min; contents in  $1.000$  ml: soybean oil  $200$  g, glycerol  $25.9$  g, egg phospholipids  $12$  g, and oleate  $0.3$  g; supplemented with Heparin-Natrium-25000-ratio-pharm; Merckle, Blaubeuren, Germany;  $0.4$  IU/kg per min) 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  $279 \pm 15$  days), which followed the same protocol, apart that saline (saline 0.9%; Fresenius Kabi, Bad Homburg, Germany;  $1.25$  ml/min) 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 (Dr Müller Gerätebau, Freital, Germany). For the measurements of serum vaspin, blood was collected in serum tubes (Bayer).

## Assays

Serum vaspin was measured using a commercially available ELISA (Adipogen, Seoul, South Korea; intra-assay coefficient of variation 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). HbA1c was measured using HA 8140 (Menarini Diagnostics, Berlin, Germany). High-density lipoprotein and total cholesterol were detected with ABX Pentra 400 (ABX Diagnostics, Montpellier, France), while low-density lipoprotein was calculated according to the Friedewald's formula.

## Statistical analysis and calculations

SPSS 16.0 (Chicago, IL, USA) was used for statistical analysis. If not stated otherwise, data are shown as means  $\pm$  S.E.M. The presence of normal distribution was analyzed 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  $\chi^2$ -test. Changes of circulating vaspin during lipid versus saline clamps were analyzed 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/m<sup>2</sup>). Regarding the 83 individuals with available EHC data, *M* value was  $4.70 \pm 0.33$  and  $4.33 \pm 0.32$  mg/kg per min 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  $0.50 \pm 0.06$  ng/ml. Surprisingly, we found the highest serum vaspin levels in normal weight subjects (lean:  $0.58 \pm 0.18$  ng/ml; normal weight:  $0.76 \pm 0.14$  ng/ml; overweight:  $0.29 \pm 0.81$  ng/ml; obese:  $0.34 \pm 0.05$  ng/ml). Furthermore, we observed significantly higher serum vaspin levels in women versus men ( $0.66 \pm 0.10$  vs  $0.29 \pm 0.04$  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)

**Table 1** Baseline characteristics of the cross-sectional study population. Means  $\pm$  S.E.M. or absolute number of subjects under medical treatment is shown.

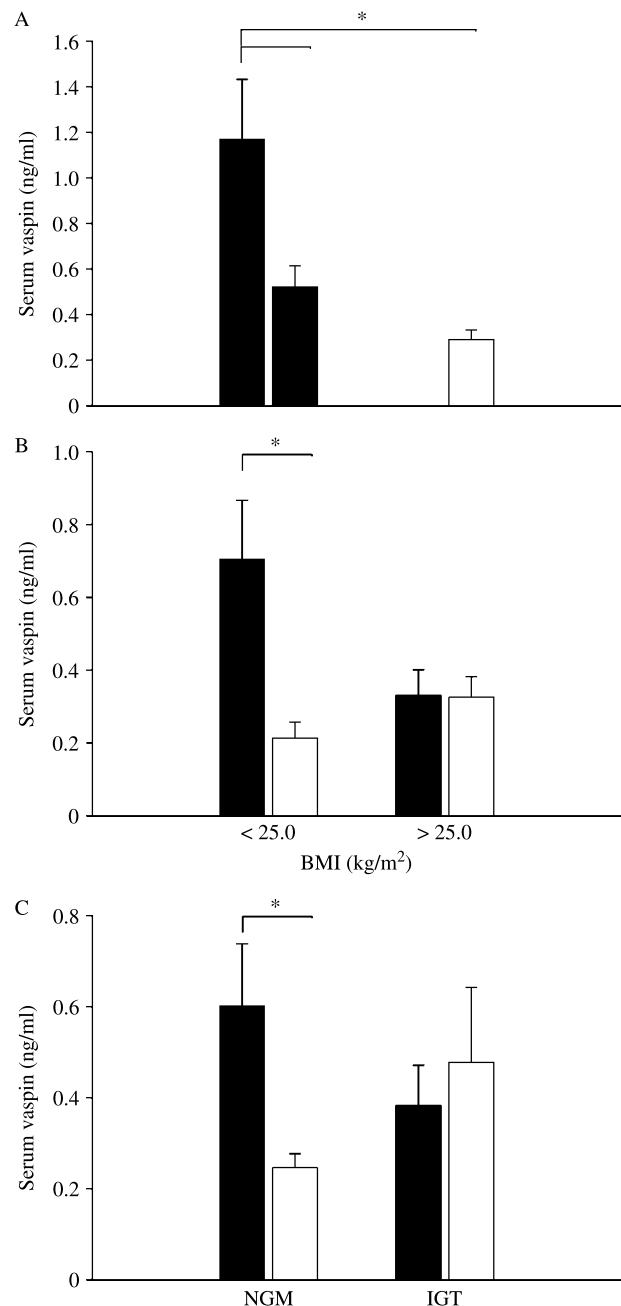
	Females	Males	P
<i>n</i>	62	46	–
Age (years)	46.6 $\pm$ 2.0	50.4 $\pm$ 2.2	0.21
BMI (kg/m <sup>2</sup> )	27.7 $\pm$ 1.2	29.3 $\pm$ 0.9	0.30
Waist circumference (cm)	87.0 $\pm$ 2.8	99.3 $\pm$ 2.3	0.001
Fasting plasma glucose (mmol/l)	4.92 $\pm$ 0.07	5.12 $\pm$ 0.07	0.08
120 min OGTT-plasma glucose (mmol/l)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

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.

( $B=0.282$ ,  $P=0.03$ ;  $R^2=0.30$ ,  $P<0.001$  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 versus females without OC usage ( $1.17 \pm 0.26$  vs  $0.52 \pm 0.09$  ng/ml,  $P=0.02$ ; Fig. 1A) and males ( $1.17 \pm 0.26$  vs  $0.29 \pm 0.04$  ng/ml,  $P=0.01$ ; Fig. 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 versus 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:  $0.06 \pm 0.01$  ng/ml; normal weight:  $0.24 \pm 0.05$  ng/ml; overweight:  $0.34 \pm 0.11$  ng/ml; obese:  $0.31 \pm 0.05$  ng/ml), while in females peak concentrations were observed in normal weight subjects (lean:  $0.31 \pm 0.11$  ng/ml; normal weight:  $0.86 \pm 0.21$  ng/ml; overweight:  $0.22 \pm 0.09$  ng/ml; obese:  $0.37 \pm 0.09$  ng/ml). The gender difference concerning circulating vaspin was abrogated ( $P=0.07$ ; Fig. 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  $<25$  vs  $\geq 25$  kg/m<sup>2</sup>. A significant sexual dimorphism was found in individuals with a BMI  $<25$  kg/m<sup>2</sup> ( $P=0.009$ ; Fig. 1B), while no differences became apparent in overweight and obese subjects ( $P=0.91$ ; Fig. 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.



**Figure 1** Gender-related distribution of vaspin serum concentrations in the cross-sectional study ( $n=108$ ). Data are means  $\pm$  S.E.M.; ■, female subjects; □, male subjects. (A) Circulating vaspin in females using OC ( $n=13$ ; left black bar) versus females without OC usage ( $n=49$ ; right black bar,  $P<0.01$ ) and versus male subjects ( $n=46$ ;  $P=0.027$ ). Females without OC usage versus 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  $<25$  kg/m<sup>2</sup>, females versus males ( $P=0.009$ ; left bars) and BMI  $\geq 25$  kg/m<sup>2</sup>, females versus males ( $P=0.90$ ; right bars). (C) Circulating vaspin of NGM ( $n=78$ , males  $n=37$ ;  $P=0.027$ ) and IGT female versus male subjects ( $n=27$ , males  $n=9$ ;  $P=0.72$ ).



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

Independent variables	$\beta$ Coefficient	<i>P</i> value
Model 1: study group with females using OC excluded ( <i>n</i> =95; <i>R</i> <sup>2</sup> =0.416, <i>P</i> <0.001)		
	log HOMA-IR	
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 ( <i>n</i> =76; <i>R</i> <sup>2</sup> =0.174, <i>P</i> =0.001)		
	log <i>M</i> value	
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.

When dividing the residual group according to IGT versus NGM (Fig. 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 vs  $\geq$ 25 kg/m<sup>2</sup> respectively), nor did we find a significant interaction of sex and IGT (*P*=0.91 and *P*=0.80 for BMI <25 vs  $\geq$ 25 kg/m<sup>2</sup> respectively).

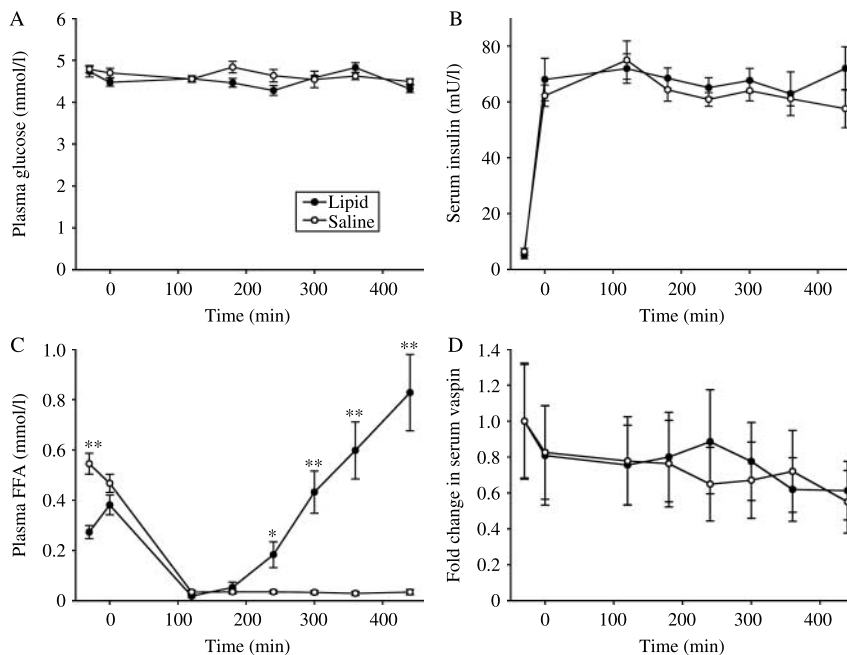
**Lipid versus saline intervention study**

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  $\pm$  0.1 mmol/l without significant differences between groups (treatment  $\times$  group effect: *P*=0.13; Fig. 2A). Steady state was reached at a mean of 137.6  $\pm$  9.0 min.

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

As expected from our experimental design, peripheral insulin sensitivity significantly decreased in lipid-exposed subjects (baseline *M* value 6.98  $\pm$  0.76 vs 5.24  $\pm$  0.91 mg/kg per min after lipid challenge; *P*=0.008) but not during the saline clamps (baseline *M* value 6.55  $\pm$  0.86 vs 6.75  $\pm$  0.59 mg/kg per min after saline; *P*=0.67). Despite euglycemic hyperinsulinemia and insulin resistance with significantly raised plasma-FFAs 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; Fig. 2D).



**Figure 2** Intervention study (*n*=10). EHC with 300 min lipid versus 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-versus saline-exposed subjects relative to the overnight fasted state. Data are means  $\pm$  s.e.m. for *n*=10 normal glucose-tolerant subjects. (B) Time  $\times$  treatment effect for insulin: baseline versus 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 versus 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 versus saline infusion, *P*=0.44.

## Discussion

Previous studies suggest a significant association of serum vaspin with insulin resistance, both as estimated using HOMA-IR (7–9) 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 subjects investigated here 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 did we detect a significant impact of IGT on circulating vaspin.

We found significantly higher circulating vaspin levels in females versus males, which is in accordance with some (6, 10), but not all previous studies (9, 11). The association of circulating vaspin with OC usage observed here 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 $\beta$ -estradiol exhibits a dose-dependent, albeit insignificant, increase in vaspin levels (8). Furthermore, a significant decrease of 17 $\beta$ -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  $\geq 25$  kg/m<sup>2</sup>. 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 substudy, 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 cohort investigated here of nondiabetic 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.

## Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

## Funding

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.

## Acknowledgements

We thank A Wagner, A Ziegenhorn, K Sprengel, and J Lin for their excellent technical assistance.

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Received 19 November 2009

Accepted 30 November 2009