Adiponectin may mediate the association between omentin, circulating lipids and insulin sensitivity: results from the KORA F4 study

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Abstract

Objective: Reduced circulating omentin levels have been reported in obesity and type 2 diabetes, but data were mostly derived from univariate analyses in small study samples. This study aimed to investigate the relationship between omentin, abnormal glucose tolerance and related metabolic factors in a large population-based cross-sectional study.

Design and methods: Serum omentin was measured by ELISA in 1092 participants of the German KORA F4 survey (2006–2008). Associations between omentin serum levels, glucose tolerance (assessed with an oral glucose tolerance test) and diabetes-related factors were estimated using logistic and linear regression models respectively.

Results: Serum levels of omentin were not related to categories of glucose tolerance. However, serum omentin was positively associated with whole-body insulin sensitivity index (ISI (composite)) and HDL cholesterol and showed inverse associations with 2-h post-load glucose, fasting insulin, homeostasis model assessment-estimated insulin resistance, BMI and triglycerides (all $P \leq 0.03$ after adjustment for age, sex and lifestyle factors). Further adjustment for BMI and/or serum lipids attenuated the associations with parameters of glucose metabolism, whereas adjustment for serum adiponectin virtually abolished all aforementioned associations. In contrast, adjustment for omentin had no effect on the positive association between adiponectin levels and ISI (composite).

Conclusions: The data from this large population-based cohort show that circulating omentin levels are associated with insulin sensitivity. Our observations further suggest that omentin acts via upregulation of adiponectin, which in turn affects lipid metabolism and thereby also indirectly enhances insulin sensitivity, but mechanistic studies are required to corroborate this hypothesis.

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Introduction

Adipose tissue is not only a source of pro-inflammatory immune mediators that contribute to the pathophysiology of type 2 diabetes, but also secretes proteins with a potential anti-inflammatory action that may protect against the development of the disease such as adiponectin, interleukin-1 receptor antagonist (IL1-RA) and omentin (also known as intelectin-1) (1). Of these factors, circulating levels of IL1-RA, the endogenous inhibitor of the potent pro-inflammatory cytokine IL1 β , are upregulated before the onset of type 2 diabetes (2, 3, 4, 5). This can be considered as futile counter-regulation of metabolic and immunological stress in the prediabetic period (2, 3). In contrast, high circulating levels of adiponectin are consistently associated with decreased risk of type 2 diabetes (6), although it has to be noted that the causal association between adiponectin and insulin sensitivity in humans as well as the regulation of adiponectin in old age and in individuals with inflammatory conditions is still poorly understood and may partly also be attributable to noninflammatory mechanisms (1).

Adiponectin levels are correlated with circulating omentin levels (7, 8), a relatively novel adipokine predominantly released by visceral adipose tissue (9), although it is also expressed in other tissues of the body (10). Several cross-sectional studies further reported that omentin levels are inversely associated with insulin resistance (assessed as homeostasis model assessment-estimated insulin resistance (HOMA-IR)) (7, 8, 11, 12, 13, 14) and decreased in individuals with impaired fasting glucose (IFG), impaired glucose tolerance (IGT), or type 2 diabetes (8, 11, 12, 14, 15, 16). Such an inverse association may be explained by the anti-inflammatory properties of omentin that have been observed in *in vitro* studies on endothelial cells and smooth muscle cells (17, 18, 19). However, data on omentin and type 2 diabetes as well as diabetes-related traits were collected in small cross-sectional studies. Therefore, it has not been investigated so far to what extent the observed link between omentin and type 2 diabetes is confounded or mediated by factors such as obesity, serum lipids, or adiponectin. To address this, we measured serum omentin levels in a large population-based cohort and assessed i) whether serum levels of omentin were inversely associated with IFG, IGT, or type 2 diabetes and with continuous metabolic factors related with type 2 diabetes (glucose, insulin, HOMA-IR, whole-body insulin sensitivity index (ISI (composite)), BMI, blood pressure and lipid levels), ii) whether associations between omentin, abnormal

glucose tolerance and related risk factors are explained by BMI or independent of obesity and iii) to what extent these associations could be explained by circulating adiponectin levels.

Subjects and methods

Study population

Data are based on the Cooperative Health Research in the Region of Augsburg (KORA) F4 study (2006–2008), the follow-up examination of the population-based KORA S4 study (1999–2001) (20). The study design and the enrolment of participants in the KORA S4 survey have been described in detail before (21). Briefly, 2656 men and women between 55 and 74 years of age were randomly selected from the region of Augsburg in the South of Germany to participate in the KORA S4 study. From the 2564 eligible subjects, 1653 (64%) completed the survey and a subsequent 1353 subjects without known diabetes successfully completed an oral glucose tolerance test (OGTT). Glucose tolerance categories were defined according to the 1999 World Health Organization diagnostic criteria (22).

The current study is based on data from the 7-year follow-up examination (F4 study) of the KORA S4 cohort, which took place in 2006–2008 and also included a standardised OGTT. Of the above-mentioned 1353 KORA S4 participants, a total of 1209 participated in the F4 follow-up examinations. We excluded individuals with unclear glucose tolerance status ($n=36$), type 1 diabetes ($n=8$), insufficient fasting time before the OGTT ($n=1$), or at least one missing variable for the statistical analyses ($n=72$), resulting in a final sample size of 1092 subjects. All participants gave written informed consent to the study, which was approved by the Ethics Committee of the Bavarian Medical Association.

Assessment of anthropometric and metabolic variables

Height, weight, waist circumference, and systolic and diastolic blood pressure were measured according to standardised protocols (21). Trained medical interviewers collected information on medical history, physical activity, smoking behaviour and alcohol consumption (20).

Cases of self-reported diabetes, as well as the date of diagnosis, were validated through contacting the participants' general practitioners. All other participants

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underwent an OGTT. After an overnight fasting period (≥ 8 h), a fasting blood sample was taken and participants ingested 75 g of anhydrous glucose orally (Dextro OGT, Boehringer Mannheim). A second blood sample was taken 2 h after the glucose challenge. The blood samples were collected without stasis. After withdrawal, the blood samples were centrifuged and kept cool at 4 °C until analysis in the Central Laboratory of the Augsburg Central Hospital. Serum glucose levels were assessed by the hexokinase method (GLU Flex, Dade Behring, Marburg, Germany). Serum insulin was determined by ELISA (Invitrogen). HOMA-IR was calculated as (fasting glucose (mmol/l) $\times$ fasting insulin (μ U/ml))/22.5. We also calculated the whole-body ISI (composite) as described by DeFronzo and Matsuda (23) using the formula ISI (composite) = $10\,000/\sqrt{(\text{fasting glucose (mmol/l)} \times 2\text{-h glucose (mmol/l)} \times \text{fasting insulin (pmol/l)} \times 2\text{-h insulin (pmol/l)} \times 36)}$. HbA1c was determined by a reverse-phase cation-exchange HPLC method (Analyzer HA 8160, Menarini, Florence, Italy).

Serum LDL and HDL cholesterol were measured by an enzymatic method (CHOD-PAP, LDL Flex and AHDH Flex, Dade Behring). Serum triglycerides were quantified by the enzymatic GPO-PAP method (TGL Flex, Dade Behring).

Kidney function was assessed by calculating the estimated glomerular filtration rate (eGFR) using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (24).

Measurement of serum concentrations of omentin and adiponectin

Serum concentrations of omentin were determined using the Human Omentin-1 ELISA from BioVendor (Brno, Czech Republic) according to the manufacturer's instructions. Intra- and inter-assay coefficients of variation (CV) were 2.0 and 4.0% respectively. Serum concentrations of total adiponectin were measured using the Human Total Adiponectin/Acrp30 Quantikine ELISA from R&D Systems (Wiesbaden, Germany) as described (25). Intra- and inter-assay CV were 3.8 and 8.0% respectively.

Statistical analyses

Participants' characteristics were stratified by quartiles of serum omentin and presented as mean $\pm$ s.d. for normally distributed variables and as median (25th/75th percentiles) for variables without a normal distribution. A general linear model (*F* test) was used to test differences in the means of continuous variables. Logistic

regression models or χ^2 tests were used to test for differences in percentages. Analyses were adjusted for age and sex except for categories of glucose tolerance and for smoking. Correlations between omentin and adiponectin (log-transformed) were additionally assessed by partial Pearson's correlation coefficients and corresponding *P* values.

Multinomial logistic regression analyses were performed to estimate odds ratios (ORs) and 95% CI for the association between serum concentrations of omentin (standardised to an increase of 1 s.d. = 171.9 ng/ml) and categories of glucose tolerance with individuals with normal glucose tolerance (NGT) as reference group. Model 1 included age (continuous) and sex. Model 2 additionally included smoking (never/ex/current), alcohol consumption (low/high) and physical activity (low/high). Model 3 included all factors from model 2 and BMI (continuous). Model 4 included all factors from model 3 and HDL cholesterol (continuous), LDL cholesterol (continuous), triglycerides (continuous), hypertension (no/yes), history of myocardial infarction (MI) (no/yes) and eGFR (continuous).

The same stepwise modelling strategy was used to calculate regression coefficients (β , standardised to a 1 s.d. increase in omentin levels) and corresponding *P* values for the association between serum omentin levels and continuous metabolic variables from linear regression analyses. In this analysis, we added a fifth model that contained all factors from model 4 and serum levels of adiponectin (continuous).

P values of <0.05 were considered to be statistically significant. All analyses were performed with SAS Software version 9.2 (SAS Institute, Cary, NC, USA).

Results

Study population

Table 1 gives the description on the study population stratified by quartiles of serum omentin levels. After adjustment for age and sex, individuals with higher omentin levels were older, more likely to be female and had a higher ISI (composite) as well as higher levels of HDL cholesterol and adiponectin. Moreover, individuals with higher omentin levels had lower BMI, 2-h glucose, fasting insulin, HOMA-IR and fasting triglyceride levels. The relationship between omentin and adiponectin was also assessed using partial Pearson's correlation coefficients adjusted for age and sex ($r=0.277$, $P<0.0001$) as well as for age, sex and BMI ($r=0.259$, $P<0.0001$).

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Table 1 Description of the KORA F4 study population stratified by quartiles of omentin serum concentrations^{a,b}.

Variable	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P
Omentin (range) (ng/ml)	67.2–402.1	402.9–488.8	489.0–580.7	580.8–2501.1	
n	273	273	273	273	
Age (years)	69.3±5.3	69.7±5.2	70.9±5.3	71.3±5.8	<0.001
Sex (% male)	60	54	49	42	<0.001
BMI (kg/m ²)	29.8±4.7	28.6±4.2	28.6±4.1	28.1±4.9	<0.001
Fasting glucose (mmol/l)	5.79±1.00	5.83±1.20	5.83±1.33	5.79±1.47	0.93
2-h glucose (mmol/l)	7.35±2.16	7.10±2.25	6.96±2.10	6.99±2.23	0.048
Fasting insulin (pmol/l)	34.2 (22.2; 69.6)	32.4 (21.6; 58.2)	29.4 (19.8; 52.8)	27.0 (18.0; 46.8)	0.002
2-h insulin (pmol/l)	328 (215; 608)	350 (189; 538)	316 (166; 503)	323 (161; 497)	0.006
HOMA-IR	1.52 (0.91; 3.02)	1.37 (0.88; 2.54)	1.19 (0.82; 2.23)	1.13 (0.70; 2.11)	0.003
ISI (composite) (1/((mmol/l)×(pmol/l)))	15.4 (7.8; 23.9)	15.0 (9.7; 26.0)	17.5 (10.4; 30.6)	18.8 (11.0; 32.6)	<0.001
HbA1c (%)	5.7±0.5	5.8±0.7	5.8±0.7	5.8±0.7	0.37
HbA1c (mmol/mol)	39±5	40±8	40±8	40±8	0.37
Glucose tolerance status					0.67
NGT (%)	51	53	55	54	
IFG (%)	6	3	7	5	
IGT (%)	17	18	15	18	
IFG/IGT (%)	4	5	5	3	
Newly diagnosed type 2 diabetes (%)	5	5	5	7	
Known type 2 diabetes (%)	17	15	13	13	
Hypertension (%) ^c	62	60	66	62	0.65
Systolic blood pressure (mmHg)	128.6±16.4	127.0±20.4	131.5±20.5	127.5±21.4	0.02
Diastolic blood pressure (mmHg)	75.5±9.3	73.7±10.3	74.2±10.3	73.1±10.3	0.25
LDL cholesterol (mmol/l)	3.59±0.86	3.66±0.93	3.65±0.90	3.55±1.00	0.42
HDL cholesterol (mmol/l)	1.33±0.31	1.44±0.36	1.44±0.37	1.54±0.37	<0.001
Fasting triglycerides (mmol/l)	1.44 (0.97; 1.91)	1.28 (0.96; 1.75)	1.31 (0.95; 1.84)	1.20 (0.89; 1.62)	0.03
Use of lipid-lowering drugs (%)	26	26	23	23	0.61
eGFR (ml/min per 1.73 m ²)	79.9 (69.2; 89.8)	80.8 (68.4; 89.0)	77.0 (67.1; 87.7)	75.8 (63.8; 87.0)	0.16
Smoking (active/ex/never) (%)	10/42/48	8/42/50	6/39/55	6/44/50	0.52
High alcohol consumption (%) ^d	14	16	20	18	0.04
Physically active (%) ^e	44	58	49	48	0.01
History of myocardial infarction (%)	5	9	3	6	0.03
Adiponectin (µg/ml)	7.8 (5.4; 11.1)	10.0 (6.4; 14.7)	10.6 (7.2; 15.1)	12.7 (9.1; 19.6)	<0.001

eGFR, estimated glomerular filtration rate; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; ISI, insulin sensitivity index; NGT, normal glucose tolerance.

^aData are given as mean±s.d., median and 25th/75th percentiles or percentages. The P values indicates differences between groups.

^bAll analyses were adjusted for age and sex except for glucose regulation and smoking.

^cBlood pressure of 140/90 mmHg or higher, or antihypertensive medication given that the subjects were aware of being hypertensive.

^dDefined as ≥40 g/day for men and ≥20 g/day for women.

^eDefined as ≥1 h sports/week in summer and in winter.

Association between serum omentin and glucose tolerance status

Supplementary Table 1, see section on supplementary data given at the end of this article, provides an overview of the study population stratified by categories of glucose tolerance. Several regression models were calculated to examine the relationship between omentin levels and glucose tolerance status. With NGT as reference category, no significant associations between omentin and IFG, IGT, combined IFG/IGT, newly diagnosed type 2 diabetes or known (previously diagnosed) type 2 diabetes were found in four models of increasing complexity (Table 2).

We also combined all groups with impaired glucose metabolism (e.g. IFG, IGT, IFG/IGT, newly diagnosed

and known type 2 diabetes; $n=513$) and compared this group with the NGT reference category ($n=579$). ORs and 95% CIs for models 1–4 were 0.91 (0.81; 1.04), 0.92 (0.81; 1.04), 0.97 (0.84; 1.11) and 0.99 (0.86; 1.13), respectively, and therefore did not point towards a significant association between serum omentin and abnormal glucose tolerance.

Association between serum omentin and quantitative risk factors of type 2 diabetes

We further examined whether omentin was associated with quantitative traits and risk factors for type 2 diabetes (Table 3). In this analysis, individuals with known type 2 diabetes were excluded, which resulted in a sample size

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Table 2 Odds ratios (OR) and 95% CI for associations between serum concentrations of omentin (per s.d.) and categories of glucose tolerance in the KORA F4 study.

Category of glucose tolerance	Model 1	Model 2	Model 3	Model 4
Known type 2 diabetes (<i>n</i> =161)	0.85 (0.69; 1.03)	0.86 (0.70; 1.05)	0.94 (0.77; 1.14)	0.96 (0.78; 1.19)
Newly diagnosed type 2 diabetes (<i>n</i> =64)	1.05 (0.82; 1.33)	1.05 (0.82; 1.34)	1.09 (0.86; 1.39)	1.14 (0.89; 1.45)
IFG + IGT (<i>n</i> =46)	0.78 (0.54; 1.12)	0.75 (0.51; 1.10)	0.83 (0.57; 1.21)	0.86 (0.58; 1.26)
IGT (<i>n</i> =186)	0.86 (0.71; 1.03)	0.86 (0.72; 1.04)	0.89 (0.74; 1.08)	0.89 (0.74; 1.08)
IFG (<i>n</i> =56)	1.18 (0.93; 1.48)	1.17 (0.93; 1.48)	1.19 (0.94; 1.52)	1.23 (0.96; 1.58)
NGT (<i>n</i> =579; reference)	1	1	1	1

IFG, impaired fasting glucose; IGT, impaired glucose tolerance; NGT, normal glucose tolerance. Model 1: adjusted for age, sex. Model 2: model 1 + smoking, alcohol, physical activity. Model 3: model 2 + BMI. Model 4: model 3 + HDL cholesterol, LDL cholesterol, triglycerides, hypertension, history of myocardial infarction.

of *n*=931. In multiple regression analyses adjusting for age and sex (model 1) and additionally for lifestyle factors (smoking, alcohol consumption, physical activity, model 2), omentin levels were positively associated with ISI (composite) and HDL cholesterol, and inversely with 2-h glucose, fasting insulin, 2-h insulin, HOMA-IR, BMI and fasting triglycerides. No associations were found for fasting glucose, HbA1c, systolic and diastolic blood pressure and LDL cholesterol. Statistical significance for the associations between omentin and fasting insulin and HOMA-IR was lost upon additional adjustment for BMI, while the associations between omentin and 2-h glucose, 2-h insulin and ISI (composite) remained statistically significant (model 3). When we adjusted for waist-to-hip ratio or waist-to-height ratio instead of BMI, results were almost identical (data not shown). Following subsequent adjustment for metabolic variables, medication, history of MI and eGFR (model 4), only the positive association between omentin and HDL cholesterol and the inverse association with fasting triglycerides remained statistically significant. Finally, we examined whether the associations between omentin, HDL cholesterol and triglycerides were independent of the relationship between adiponectin and these lipid variables. Whereas adiponectin was significantly associated with both HDL cholesterol ($P<0.0001$) and triglycerides ($P<0.0001$), adding adiponectin to model 4 resulted in a loss of statistical significance for the associations between omentin and HDL cholesterol and triglycerides.

In addition to the *a priori* defined models 1–5, we investigated which of the additional covariables in models 4 and 5 had the most pronounced effect on the association between omentin and ISI (composite) as main surrogate index of insulin sensitivity by adding each of these covariables separately to model 3. Model 3 yielded an effect estimate (β (95% CI)) of 0.07 (0.01; 0.12). This effect

estimate was almost unaltered by adjustment for LDL cholesterol (0.07 (0.01; 0.12)), hypertension (0.07 (0.01; 0.12)), history of MI (0.06 (0.01; 0.12)) or eGFR (0.07 (0.01; 0.12)), lost significance when adding HDL cholesterol (0.04 (–0.01; 0.10)) or triglycerides (0.05 (–0.001; 0.11)) and was almost entirely abolished by adiponectin (0.004 (–0.05; 0.06)).

Finally, we modified the study question and analysed the association between adiponectin and ISI (composite). This approach yielded an effect estimate of 0.039 (0.030; 0.048) for an analysis that considered all covariables from model 3. This highly significant association was not attenuated by additional adjustment for omentin (0.039 (0.029; 0.048)).

Discussion

This large study of an elderly, population-based sample found that serum omentin does not associate with abnormal glucose tolerance, but shows positive associations with insulin sensitivity (ISI (composite)) and correlates inversely with 2-h glucose, fasting insulin, 2-h insulin and HOMA-IR. Omentin also associates positively with HDL cholesterol and inversely with triglycerides. These associations were independent of kidney function assessed by eGFR. Importantly, circulating adiponectin levels almost entirely explain the associations between omentin and insulin sensitivity as well as lipids. Collectively, these observations suggest that the associations between omentin on the one hand and insulin sensitivity as well as lipids on the other hand are mainly explained by adiponectin. Thus, our population-based study shows that most of the previously reported relations between omentin, obesity and type 2 diabetes may be ascribed to the regulatory effects on adiponectin (and to a lesser extent on lipid) levels as putative intermediaries.

Table 3 Association between serum omentin concentrations and continuous metabolic traits in the KORA F4 study. β coefficients with 95% CIs and P values standardised to 1 s.d. increases in omentin levels from linear regression analyses. Significant associations ($P < 0.05$) are highlighted by bold print.

Variable	Model 1		Model 2		Model 3		Model 4		Model 5	
	β (95% CI)	P	β (95% CI)	P	β (95% CI)	P	β (95% CI)	P	β	P
Fasting glucose (mmol/l)	-0.02 (-0.06; 0.02)	0.28	-0.02 (-0.06; 0.02)	0.27	-0.01 (-0.05; 0.03)	0.63	0.00 (-0.04; 0.04)	0.92	0.02 (-0.02; 0.06)	0.40
2-h glucose (mmol/l)	-0.19 (-0.33; -0.05)	0.01	-0.18 (-0.32; -0.04)	0.01	-0.14 (-0.28; -0.01)	0.04	-0.11 (-0.24; 0.03)	0.12	-0.06 (-0.20; 0.08)	0.41
HbA1c (%)	0.00 (-0.03; 0.02)	0.77	0.00 (-0.02; 0.02)	0.99	0.00 (-0.02; 0.03)	0.69	0.01 (-0.01; 0.03)	0.38	0.02 (-0.004; 0.04)	0.11
Ln (fasting insulin) (pmol/l)	-0.07 (-0.14; -0.01)	0.02	-0.07 (-0.13; -0.01)	0.02	-0.05 (-0.10; 0.01)	0.10	-0.03 (-0.09; 0.03)	0.27	0.00 (-0.06; 0.06)	0.92
Ln (2-h insulin) (pmol/l)	-0.08 (-0.14; -0.03)	0.005	-0.08 (-0.14; -0.02)	0.01	-0.06 (-0.11; -0.003)	0.04	-0.04 (-0.09; 0.01)	0.15	0.00 (-0.06; 0.05)	0.98
Ln (HOMA-IR)	-0.08 (-0.14; -0.02)	0.02	-0.08 (-0.14; -0.02)	0.02	-0.05 (-0.11; 0.01)	0.10	0.04 (-0.01; 0.10)	0.10	0.01 (-0.05; 0.06)	0.82
Ln ISI (composite)	0.10 (0.04; 0.16)	0.002	0.09 (0.03; 0.15)	0.003	0.07 (0.01; 0.12)	0.02	-0.03 (-0.09; 0.03)	0.27	0.00 (-0.06; 0.06)	0.98
BMI (kg/m ²)	-0.31 (-0.59; -0.03)	0.03	-0.30 (-0.57; 0.02)	0.03	NA	NA	-0.12 (-0.37; 0.14)	0.37	-0.05 (-0.31; 0.22)	0.74
Systolic blood pressure (mmHg) ^a	-0.05 (-1.31; 1.21)	0.94	-0.10 (-1.36; 1.16)	0.88	-0.06 (-1.33; 1.20)	0.92	-0.02 (-1.29; 1.25)	0.98	0.41 (-0.89; 1.72)	0.53
Diastolic blood pressure (mmHg) ^a	-0.59 (-1.22; 0.04)	0.07	-0.60 (-1.24; 0.03)	0.06	-0.58 (-1.21; 0.06)	0.07	-0.54 (-1.17; 0.10)	0.10	-0.36 (-1.01; 0.29)	0.28
LDL cholesterol (mmol/l) ^b	-0.03 (-0.09; 0.03)	0.27	-0.03 (-0.09; 0.03)	0.34	-0.03 (-0.09; 0.03)	0.35	-0.03 (-0.08; 0.03)	0.37	-0.01 (-0.07; 0.05)	0.70
HDL cholesterol (mmol/l) ^b	0.05 (0.03; 0.08)	<0.001	0.05 (0.03; 0.07)	<0.001	0.04 (0.02; 0.06)	<0.001	0.04 (0.02; 0.06)	<0.001	0.01 (-0.01; 0.03)	0.22
Ln (fasting triglycerides) (mmol/l) ^b	-0.05 (-0.08; -0.02)	0.003	-0.05 (-0.08; -0.01)	0.004	-0.04 (-0.07; -0.01)	0.02	-0.04 (-0.07; -0.01)	0.01	-0.01 (-0.04; 0.02)	0.55

NA, not applicable. Model 1: adjusted for age and sex. Model 2: model 1 + smoking, alcohol, physical activity. Model 3: model 2 + BMI. Model 4: model 3 + HDL cholesterol, LDL cholesterol, triglycerides, hypertension, history of MI, eGFR. Model 5: model 4 + adiponectin.

^aHypertension was replaced by use of antihypertensive drugs in models 4 and 5.

^bHDL cholesterol, LDL cholesterol and triglycerides were replaced by use of lipid-lowering drugs in models 4 and 5.

Omentin and glucose metabolism

This is the first population-based study ($n=1092$ participants) addressing the association between omentin and abnormal glucose tolerance. Using a series of regression models with extensive adjustment for potential confounders and intermediaries, we found no independent associations between omentin and glucose tolerance. Our data differ from previous studies reporting an inverse relation between omentin levels and type 2 diabetes (8, 12, 15, 16) and IFG and/or IGT (8, 12). However, these studies were based on selected groups of patients and controls, and data from only one of these studies were adjusted for confounders or potential intermediaries (8). However, one should note that the mean HbA1c level in subjects with type 2 diabetes was relatively high (9.7%) in this study, indicating poor glucometabolic control, whereas in our study population, mean HbA1c in patients with known or newly diagnosed type 2 diabetes was 6.76 and 6.10%, respectively, which may explain the discrepancy of the study results.

When examining additional continuous traits, which define glucose tolerance or are associated with insulin resistance, we found no associations between omentin and fasting glucose or HbA1c. However, omentin levels were associated with the OGTT-based ISI (composite) and inversely related to 2-h glucose, fasting and 2-h insulin and HOMA-IR. These associations were independent of age, sex and lifestyle factors, but further adjustment for BMI, lipids or adiponectin abolished statistical significance. Notably, the inverse relation between omentin levels on the one hand and 2-h glucose, 2-h insulin and ISI (composite), which represents an OGTT-derived measure of insulin sensitivity that is more closely related to clamp-measured insulin sensitivity than HOMA-IR (23), on the other hand remained significant after adjustment for BMI, but not after adjustment for lipids or adiponectin. Some (10, 26), but not other (7, 11, 27, 28), studies reported inverse associations between omentin and fasting glucose in univariate analyses. One study reported inverse associations with fasting glucose, 2-h glucose and HbA1c in univariate, but not in multivariate analyses (12). Results were also conflicting for fasting insulin and HOMA-IR with several studies observing inverse associations for insulin (27) and HOMA-IR (8, 10, 11, 12, 14, 27), whereas others reported no significant relationships for insulin (7, 11, 12) or HOMA-IR (7, 26). Overall, the comparison of study results is hampered by the differences in sample selection, sample size and statistical

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analyses. However, it is striking that most studies failed to detect an association between omentin and fasting glucose. Moreover, most studies have analysed associations with HOMA-IR, but only three (7, 12, 14) adjusted for covariables. Among these studies, one did not observe a significant association in an Amish population (7), whereas the two others found inverse associations in Chinese samples (12, 14), but their multivariate models did not consider lipid levels or adiponectin as potential mediators. In this context, three small intervention studies are of interest, which examined the association between changes in omentin and HOMA-IR in overweight/obese individuals resulting from weight loss following a hypocaloric diet for 4 months (29), aerobic exercise for 3 months (13) or bariatric surgery (30). Both the hypocaloric diet study and the exercise study reported significant increases in omentin, which associated with an improvement of HOMA-IR, but these interventions also decreased LDL cholesterol and triglyceride levels (13, 29). Bariatric surgery induced a substantial weight loss and a decrease in HOMA-IR within 3 months (means at baseline and after 3 months 3.6 and 1.4), but no notable change in plasma omentin levels (30). Interestingly, the levels of total, LDL and HDL cholesterol as well as triglyceride levels remained also unchanged during these 3 months. However, omentin levels were increased at the 3-year follow-up concomitantly with a significant increase in HDL cholesterol (+36%) and a 33%, albeit non-significant, decrease in triglycerides. Therefore, there is increasing evidence that previous reports of omentin as a novel biomarker for insulin resistance may have been based on indirect effects owing to alterations in lipid metabolism in their underlying analyses, indicating that the relationship between omentin and lipid metabolism needs further attention as discussed in more detail below. Unfortunately, adiponectin levels were not reported in these three studies.

Omentin and BMI

One finding that triggered the interest in omentin several years ago was the observation that its circulating concentrations correlated with adiponectin levels (7) and were – similarly to adiponectin, but in contrast to other adipokines – downregulated in obesity (7, 8, 10, 12, 14, 15). We confirmed the inverse association between serum omentin and BMI, but extended previous studies by the observation that this association was largely due to lipid levels and adiponectin. The aforementioned study

on bariatric surgery corroborates the notion that omentin and BMI may not be causally related because a 17% reduction in BMI corresponding to a weight loss of 22.8 kg was not associated with any change in omentin during the first 3 months of follow-up (30).

Omentin, lipid levels and adiponectin

We found a positive association between omentin levels and HDL cholesterol, an inverse association with triglyceride levels and no association with LDL cholesterol. These associations were independent of age, sex, BMI, lifestyle factors, hypertension, history of MI and the use of lipid-lowering drugs. Our findings go beyond previous reports that were based on smaller samples and on univariate analyses. Most studies observed a positive association between omentin and HDL cholesterol (7, 11, 15, 26, 28, 31), whereas the inverse association with triglycerides was found only in some (26, 28), but not all (7, 11, 15, 16) samples.

A central observation of our study is that the associations between omentin and lipids as well as insulin sensitivity, which are notably similar to those between adiponectin and these two parameters, were virtually abolished when adiponectin entered the multivariate model. This finding is in line with the hypothesis that omentin upregulates adiponectin release which in turn favourably affects lipid levels and thereby improves insulin sensitivity. The fact that adjustment for omentin did not attenuate the association between adiponectin and insulin sensitivity argues against the alternative hypothesis that adiponectin upregulates omentin which then acts as mediator and influences lipid and glucose metabolism. However, it should be emphasised here that our study is cross-sectional and does not allow drawing firm conclusions. Nevertheless, it does help to generate hypotheses that can be tested in further experiments.

Indeed, the precise mechanisms that link adiponectin, omentin and lipid or glucose metabolism remain obscure. Although *in vitro* studies show that omentin exerts direct anti-inflammatory and insulin-sensitising effects on multiple cell types (9, 15, 17, 18, 19), there is only limited knowledge of the signalling cascade utilised by this soluble lectin. Although it is known that omentin binds to the iron-binding protein lactoferrin (32), which has been linked with insulin resistance and altered glucose tolerance (33), a more detailed understanding of the molecular mechanism of omentin action will undoubtedly help to gain further insights into the

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triangular relationship between adiponectin, omentin and lipid metabolism. Importantly, we are not aware of any study that analysed whether omentin upregulates adiponectin expression or release, which should be tested in order to corroborate our hypothesis.

Adiponectin has recently been described as downstream effector of fibroblast growth factor-21 (FGF21) (34). This finding raises the questions whether omentin and FGF21 may be correlated and whether they interact in the regulation of adiponectin. However, data on such an interaction are neither available in our study nor in the published literature.

Strengths and limitations

Our study has several strengths and limitations that need to be mentioned. Of note, our study represents the so far largest and only population-based sample in which the association between omentin, glucose metabolism and diabetes-related factors was analysed. The study sample was well-phenotyped, including an OGTT. We adjusted our observations for multiple covariables. In addition, data for adiponectin were available allowing us to interpret our findings regarding potential mediators of the effects of omentin.

Limitations of our study include the cross-sectional design, which allows us to generate testable hypotheses with respect to potential metabolic effects and putative intermediaries (adiponectin and lipids), but not to draw firm conclusions with respect to mechanistic effects. We used the HOMA-IR and ISI (composite) as surrogate measures of insulin resistance rather than the gold standard measure, the hyperinsulinaemic-euglycaemic clamp. Further limitations are the age range and the homogeneous ethnicity of our study population, so that our data are not generalisable to younger and to non-Caucasian populations. We also refrained from correction for multiple testing because the anthropometric and metabolic outcome variables are not independent so that correction using, for example, the method of Bonferroni would be overly conservative.

Conclusions

In our population-based study sample, we observed that insulin sensitivity assessed using ISI (composite) and HDL cholesterol levels was positively associated and 2-h glucose, fasting and 2-h insulin, HOMA-IR and triglycerides were inversely associated with omentin levels. These associations were partially explained by BMI and/or lipid

levels, but adjustment for adiponectin had the most attenuating effect on these associations. In contrast, adjustment for omentin did not affect the association between adiponectin and insulin sensitivity. Collectively, our data are in line with the hypothesis that circulating levels of omentin regulate adiponectin levels, which in turn improve lipid metabolism and may affect glucose metabolism indirectly by adiponectin and lipid-mediated processes. The identification of a membrane-bound omentin receptor and subsequent signalling cascades as well as studies on the interaction between omentin and adiponectin will help to better understand the physiological role of omentin and its impact on metabolic regulation.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/EJE-14-0879>.

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.

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Author contribution statement

C Herder designed the study, obtained funding, drafted statistical analysis plans and wrote the manuscript. D M Ouwens and M Carstensen obtained funding, contributed to study design, contributed to discussion, reviewed and edited the manuscript. B Kowall conducted data analysis and reviewed the manuscript. C Huth, C Meisinger and W Rathmann contributed to study organisation, provided data and reviewed the manuscript. M Roden and B Thorand contributed to study organisation, provided data, contributed to discussion and reviewed and edited the manuscript.

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