

Omentin Plasma Levels and Gene Expression Are Decreased in Obesity

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Central obesity and the accumulation of visceral fat are risk factors for the development of type 2 diabetes and cardiovascular disease. Omentin is a protein expressed and secreted from visceral but not subcutaneous adipose tissue that increases insulin sensitivity in human adipocytes. To determine the impact of obesity-dependent insulin resistance on the regulation of two omentin isoforms, gene expression and plasma levels were measured in lean, overweight, and obese subjects. Omentin 1 was shown to be the major circulating isoform in human plasma. Lean subjects had significantly higher plasma omentin 1 levels than obese and overweight subjects. In addition, higher plasma omentin 1 levels were detected in women compared with men. Plasma omentin 1 levels were inversely correlated with BMI, waist circumference, leptin levels, and insulin resistance as measured by homeostasis model assessment and positively correlated with adiponectin and HDL levels. Both omentin 1 and omentin 2 gene expression were decreased with obesity and were highly correlated with each other in visceral adipose tissue. In summary, decreased omentin levels are associated with increasing obesity and insulin resistance. Therefore, omentin levels may be predictive of the metabolic consequences or co-morbidities associated with obesity. *Diabetes* 56:1655–1661, 2007

Obesity is a chronic pathological condition and a risk factor for type 2 diabetes and cardiovascular disease (1–4). Several studies have shown that visceral obesity in particular is strongly associated with insulin resistance, hyperglycemia, dyslipidemia, and hypertension. Subcutaneous fat deposition has also been associated with decreased risk of cardiovas-

cular disease in some studies (5–9). In light of the divergent pathological consequences of differences in adipose tissue distribution, it is of great interest to resolve the molecular differences between visceral and subcutaneous adipose tissue depots. Although anatomical location and vascularization are clearly different (10), the molecular basis of differences in metabolism and secretory profile between visceral (omental) and subcutaneous adipose tissues and their impact on whole-body physiology are still not totally understood.

Omentin is a newly identified secretory protein that is highly and selectively expressed in visceral adipose tissue relative to subcutaneous adipose tissue (11–13). Omentin has been identified in other tissues at lower expression levels and named intelectin (14), intestinal lactoferrin receptor (15), or endothelial lectin (16). It is expressed in intestinal Paneth cells (17), endothelial cells (16), and visceral adipose stromal-vascular cells (11). In vitro studies have shown that omentin increases insulin signal transduction by activating the protein kinase Akt/protein kinase B and enhancing insulin-stimulated glucose transport in isolated human adipocytes. Thus, omentin may play a paracrine or endocrine role in modulating insulin sensitivity.

A homolog of omentin has been identified that shares 83% amino acid identity with omentin/intelectin (16) and will be referred to as omentin 2. The two omentin genes, omentin 1 and omentin 2, are localized adjacent to each other in the 1q22-q23 chromosomal region (18), which has been previously linked to type 2 diabetes in several populations (19–23).

Based on the preferential expression of omentin 1 in visceral fat, the presence of omentin in the circulation, and its potential role as an insulin sensitizer, we evaluated the association of omentin levels with measures of obesity, insulin resistance, and related features. We developed and validated a quantitative Western blotting assay that allowed us to measure omentin levels in the plasma. We found that omentin 1 is the major circulating form of omentin and that its plasma levels and adipose tissue gene expression are decreased with obesity. In addition, plasma omentin 1 is positively correlated with plasma adiponectin and HDL and negatively correlated with homeostasis model assessment (HOMA), a measure of insulin resistance.

RESEARCH DESIGN AND METHODS

These studies involved healthy volunteers. Subjects who had malignant disease, diabetes, or major renal, hepatic, and/or thyroid dysfunction were excluded. None were on hormonal replacement therapy. The study protocols were approved by the Institutional Review Boards for Human Subjects

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AFDS, Amish Family Diabetes Study; HOMA, homeostasis model assessment; pI, isoelectric point.

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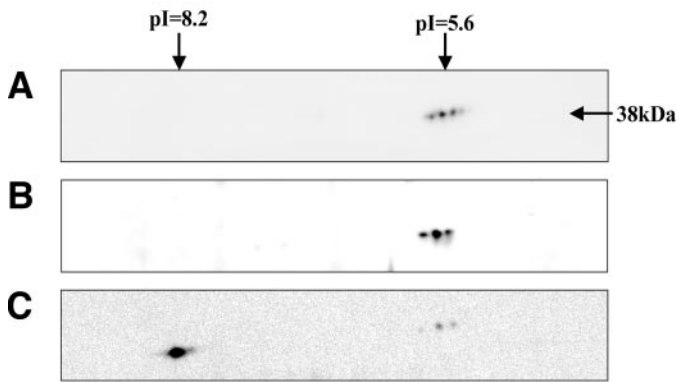


FIG. 1. Identification of omentin 1 as the major omentin isoform in human plasma. Purified omentin 1 (A), human plasma (B), and human plasma plus exogenous omentin 2 (C) were subjected to two-dimensional gel electrophoresis and Western blotting with anti-human omentin monoclonal antibody. Spots indicate immunoreactive omentin 1 and 2 with pIs of ~ 5.6 and 8.2 , respectively.

Research at University of Maryland and Johns Hopkins University. Informed consent was obtained from all subjects.

Effect of obesity on omentin gene expression. Human visceral (omental) adipose tissues were obtained from 20 subjects (2 men and 18 women) over a range of age (24–73 years) and BMI (21.5–66.6 kg/m²) undergoing intra-abdominal surgery at the University of Maryland Medical Center or The Johns Hopkins Bayview Medical Center. After excision, tissue was immediately frozen in liquid nitrogen and stored at -80°C for subsequent RNA extraction and quantitative RT-PCR analysis. In a subgroup of these subjects ($n = 14$, 2 men and 12 women) over a range of age (24–50 years) and BMI (38.1–66.6 kg/m²), blood samples were collected after overnight fasting on a visit before the day of the surgery. Plasma was separated and stored at -80°C for subsequent quantification of omentin levels.

Effect of obesity on human plasma omentin levels. A subset of 94 healthy subjects (54 women and 40 men) from the previously described Amish Family Diabetes Study (AFDS) (24) were selected as 47 sibpairs that best fit the criterion of age-matched (within 5 years) and sex-matched BMI discordance (>3.0 kg/m² difference). The BMI-discordant sibpair design was chosen to maximize the power to detect association with BMI. One subject did not fall within the detection limits of the assay, and two subjects were considered outliers (>3 SD from the mean). Consequently, these three subjects were eliminated from all analyses. Therefore, 44 pairs were used in the sibpairs analysis. For additional analyses, the participants ($n = 91$, 52 women and 39 men) were divided into three groups: 1) lean ($n = 39$; BMI <25 kg/m²), 2) overweight ($n = 30$; 25 kg/m² \leq BMI <30 kg/m²), and 3) obese ($n = 22$; BMI ≥ 30.0 kg/m²).

Identification of omentin 1 and omentin 2 in human plasma by two-dimensional gel electrophoresis. Human plasma samples, purified recombinant omentin 1 (11), and omentin 2 were separated by two-dimensional gel electrophoresis followed by Western blotting with anti-omentin monoclonal antibodies. Omentin 2 cDNA was subcloned into pcDNA3 (Invitrogen, Carlsbad, CA) and transfected into HEK-293A cells using Lipofectamine Plus (Invitrogen). Stably transfected cells were selected with 300 $\mu\text{g}/\text{ml}$ G418 (Invitrogen). Omentin 2-expressing cells were cultured in 10% fetal bovine serum/Dulbecco's modified Eagle's medium until 80% confluent and then switched to serum-free medium for 5 days. Conditioned medium containing omentin 2 was then harvested and concentrated using Centricon centrifugal concentrators (Millipore, Billerica, MA). Plasma samples, omentin 1, and/or omentin 2 standards were diluted in rehydration buffer (Bio-Rad, Hercules, CA). First-dimension isoelectric focusing was conducted using immobilized pH gradient strips (pH 3–10, 11 cm; Bio-Rad) in a Bio-Rad IEF Cell. After focusing, strips were equilibrated, and they were subjected to 10% SDS-PAGE. Proteins were then transferred to Immobilon-polyvinylidene fluoride membrane (Millipore), blocked with Starting Block Solution (Pierce, Rockford, IL) plus 0.1% Tween 20 (Pierce), and incubated with 3G1B3, a human omentin-specific monoclonal primary antibody (11), followed by horseradish peroxidase-conjugated anti-mouse secondary antibody (KPL, Gaithersburg, MD). Immunoreactive spots were visualized by chemiluminescent detection with the Femto-West kit (Pierce) on a Fluorchem 8000 chemiluminescent/fluorescent imager (Alpha Innotech, San Leandro, CA) (Fig. 1).

Quantification of human plasma omentin levels. The quantification of human plasma omentin levels was performed in three steps. Initially, purified

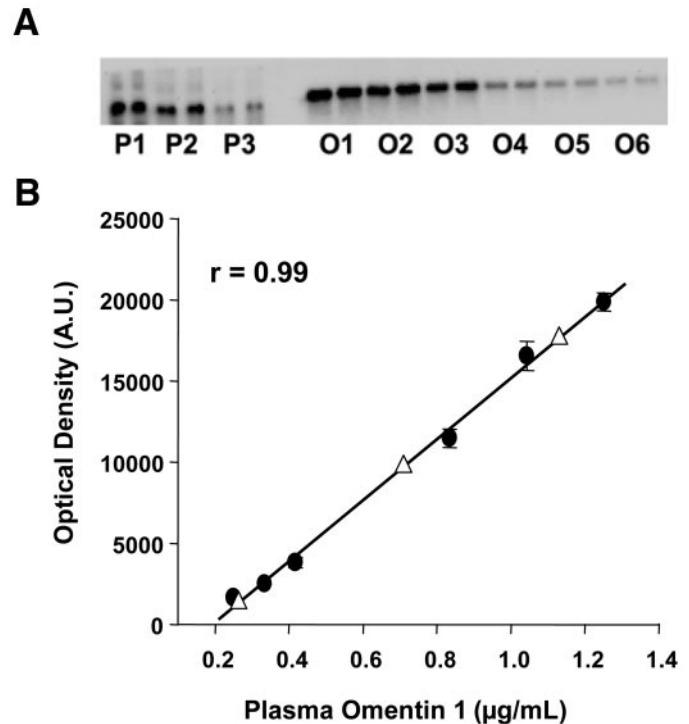


FIG. 2. Quantification of omentin 1 levels in human plasma samples. A: Plasma samples ("standard plasma") from human subjects (marked P1, P2, and P3) and purified omentin 1 at six different concentrations (marked O1–O6) were electrophoresed in duplicate on 10% SDS-PAGE gel followed by Western blotting with anti-omentin antibodies. B: The standard curve was constructed by plotting known omentin concentrations (●) versus their optical densities in arbitrary units. Omentin concentrations in plasma (△) were extrapolated from this standard curve.

omentin was quantitated against a BSA standard curve using 10% SDS-PAGE. After electrophoresis, protein bands were visualized with Sypro Ruby fluorescent protein stain (Bio-Rad). A standard curve was constructed using the log-transformed BSA concentrations versus the band intensities, and purified omentin 1 was quantitated by extrapolation from this curve. In the next step, a subset of plasma samples was analyzed by quantitative Western blotting (described above) using the previously quantified purified omentin 1 as a standard. A standard curve was constructed using the purified omentin concentrations versus the optical densities, and "standard plasma samples" were quantitated by extrapolation from this curve (Fig. 2). In the last step, "standard plasma samples" were then used to quantify the rest of the samples by quantitative Western blotting. A standard curve was constructed using the "standard plasma" concentrations versus the optical densities, and plasma samples were quantitated by extrapolation from this curve. Fluorescent or immunoreactive band intensities were visualized and quantified on a Fluorchem 8000 chemiluminescent/fluorescent imager (Alpha Innotech). Duplicate measurements differing by $>10\%$ were rejected, and the samples were retested.

Quantification of human plasma adiponectin levels. Total adiponectin levels were determined in fasting plasma samples by a commercial radioimmunoassay kit (Linco Research, St. Louis, MO). The samples were analyzed in duplicate and measured on an automated Packard Cobra-II Auto Gamma counter (Perkin-Elmer, Wellesley, MA). The duplicate measurements with counts differing by $>10\%$ were rejected, and the samples were retested.

Quantitative real-time PCR analysis. Omentin 1 and omentin 2 mRNA levels were measured by real-time quantitative RT-PCR in a LightCycler 480 (Roche Applied Science, Indianapolis, IN). Total RNA was extracted from omental adipose tissue samples with Trizol (Invitrogen). Total RNA was reverse transcribed using the Roche Transcriptor cDNA kit. cDNA (50 ng) was quantified using LightCycler 480 Probes Master kit (Roche Applied Science) and Taqman probe/primer sets (omentin 1, Hs00214137_m1; omentin 2, Hs00365614_m1; Applied Biosystems, Foster City, CA). 18S mRNA (18S RNA, Hs999999_sl) was used as an internal control for normalization. Each sample was run in duplicate. Duplicates exhibiting SDs of >0.25 cycle threshold were repeated. Copy numbers of the specific mRNAs were obtained using standard curves generated with omentin 1-containing (11) and omentin 2-containing (pcDNA3) plasmids. Normalized gene expression values were obtained using LightCycler Relative Quantification software (LightCycler 480; Roche Applied Science).

TABLE 1
Demographic and clinical characteristics of the AFDS subjects divided into lean, overweight, and obese groups

Variable	Lean	Overweight	Obese
<i>n</i>	39	30	22
Sex (men/women)	18/21	13/17	8/14
Age (years)	43 ± 16	43 ± 12	46 ± 11
BMI (kg/m ²)	21.9 ± 2.1	27.1 ± 1.5*	32.6 ± 2.2*
Waist circumference (cm)	82.3 ± 8.3	91.5 ± 8.2*	100.2 ± 6.8*
Insulin (pmol/l)	50.6 ± 13.7	65.6 ± 23.1*	74.8 ± 32.3*
Glucose (mmol/l)	4.9 ± 0.4	5.0 ± 0.4*	5.1 ± 0.3
HOMA index	1.8 ± 0.5	2.5 ± 0.9*	2.8 ± 1.2*
Triglycerides (mmol/l)	0.67 ± 0.33	0.89 ± 0.37*	1.03 ± 0.38*
Total cholesterol (mmol/l)	5.4 ± 1.1	5.3 ± 1.0	5.4 ± 1.1
HDL cholesterol (mmol/l)	1.5 ± 0.4	1.3 ± 0.3*	1.2 ± 0.3*
LDL cholesterol (mmol/l)	3.6 ± 1.0	3.7 ± 0.8	3.8 ± 1.0
Systolic blood pressure (mmHg)	112.3 ± 12.0	121.8 ± 13.0*	122.9 ± 13.7*
Diastolic blood pressure (mmHg)	74.4 ± 7.3	78.7 ± 9.7*	79.0 ± 7.5*
Adiponectin (μg/ml)	18.1 ± 6.3	12.2 ± 5.0*	12.4 ± 5.5*
Leptin (ng/ml)	4.3 ± 3.4	9.1 ± 6.2*	18.0 ± 11.4*
Omentin (μg/ml)	0.37 ± 0.12	0.31 ± 0.11*	0.31 ± 0.11*

Data are means ± SD. **P* < 0.05 (adjusted for family structure).

Statistical analysis. To adjust for family structure, variance component analysis as implemented in SOLAR (Sequential Oligogenic Linkage Analysis Routines), which allows the inclusion of a kinship matrix to allow for residual intra-pair correlations between relatives, was used for all analyses involving the association of circulating omentin levels with other obesity- and metabolic syndrome-related traits in Amish subjects (25). Before analysis, data were tested for normality of distribution by the Shapiro-Wilk test. Consequently, HOMA, leptin, insulin, and triglyceride levels were natural log-transformed to obtain a normal distribution. Spearman rank correlations were used to estimate relationships between omentin gene expression and other quantitative variables in the non-Amish (unrelated) subjects using SAS 9.1 software package (SAS, Cary, NC).

RESULTS

Human plasma samples contain detectable omentin 1 levels but not omentin 2 by two-dimensional Western blotting. Because two highly homologous isoforms of omentin/intelectin exist (16,18) and may contribute to total circulating immunologically detectable omentin levels, two-dimensional Western blots were used to determine specificity of the 3G1B3 monoclonal antibody (11) and the relative abundance of omentin 1 and 2 in plasma. Two-dimensional gel electrophoresis of omentin 1 purified from conditioned medium (11) followed by Western blotting resolved three spots with isoelectric point (pI) ≈ 5.6 and an apparent molecular weight of 38 kDa (Fig. 1A). All immunoreactive species, most likely resulting from post-translational modification, were confirmed to be omentin 1 by matrix-assisted laser desorption/ionization/time of flight mass spectrometry analysis (data not shown). A similar pattern was observed when human plasma samples were loaded (Fig. 1B). When omentin 2-conditioned medium was analyzed alone, only one spot with a slightly lower molecular weight and a pI of 8.2 was observed (data not shown). When omentin 2-conditioned medium was added to human plasma, three spots with pI ≈ 5.6 plus one spot with pI ≈ 8.2 were observed in a similar molecular weight range (Fig. 1C). The experimentally determined pIs were identical to the predicted pI values for omentin 1 and 2 based on amino acid sequence (26). These data suggest that the 3G1B antibody recognizes both omentin 1 and 2 and that omentin 1 is present in human plasma but that omentin 2 is either absent or below the limit of detection of the quantitative Western blot.

Human plasma omentin 1 levels negatively correlate with obesity and insulin resistance markers. Metabolic and demographic characteristics of the AFDS subjects adjusted for family structure are presented in Table 1. Plasma omentin 1 levels, determined by quantitative Western blotting, were significantly higher in the leaner members of the BMI-discordant sibpairs than in their heavier siblings (paired *t* test, *n* = 44 sibpairs, *P* = 0.002). After regrouping subjects into established BMI categories, a variance component analysis, adjusted for age, sex, and family structure, was performed to study the differences between lean and overweight subjects and between lean and obese subjects. Plasma omentin 1 levels (SE) were shown to be significantly higher in the lean group (0.37 ± 0.02 μg/ml, *n* = 39) than in overweight (0.31 ± 0.02 μg/ml, *n* = 30, *P* = 0.0009) and obese (0.31 ± 0.02 μg/ml, *n* = 22, *P* = 0.009) groups. In a sex-stratified analysis in which adiposity level was coded on a linear scale (0, lean; 1, overweight; and 2, obese), in both women and men, increasing levels of adiposity were associated with decreased plasma omentin 1 levels (*P* = 0.006 and 0.03, respectively; Fig. 3). A sex difference in circulating omentin 1 levels was observed when comparing all women to all men, adjusted for BMI category (0.36 ± 0.03 vs. 0.31 ± 0.02 μg/ml, *P* = 0.03), and when comparing lean women with lean men (0.41 ± 0.03 vs. 0.33 ± 0.02 μg/ml, *P* = 0.03; Fig. 3). Significant correlations based on variance components analysis adjusted for sex, age, and family structure were found between plasma omentin 1 levels and BMI (*r* = -0.34, *P* = 0.0007), waist circumference (*r* = -0.35, *P* = 0.0005), Ln HOMA index (*r* = -0.33, *P* = 0.002), Ln leptin (*r* = -0.41, *P* = <0.0001), Ln fasting insulin (*r* = -0.31, *P* = 0.003), adiponectin (*r* = 0.29, *P* = 0.005), and HDL levels (*r* = 0.31, *P* = 0.003) (Fig. 4; Table 2). The correlation coefficients were calculated by taking the square root of the percent reduction in variance in omentin when adding in the specified predictor variable in a model already containing age and sex. When the variance components analysis was adjusted for BMI or waist circumference as well as sex, age, and family structure, only leptin retained a significant although reduced correlation with omentin 1 levels (*r* = -0.25, *P* = 0.02 and *r* = -0.24, *P* = 0.02, respectively) (Table 2).

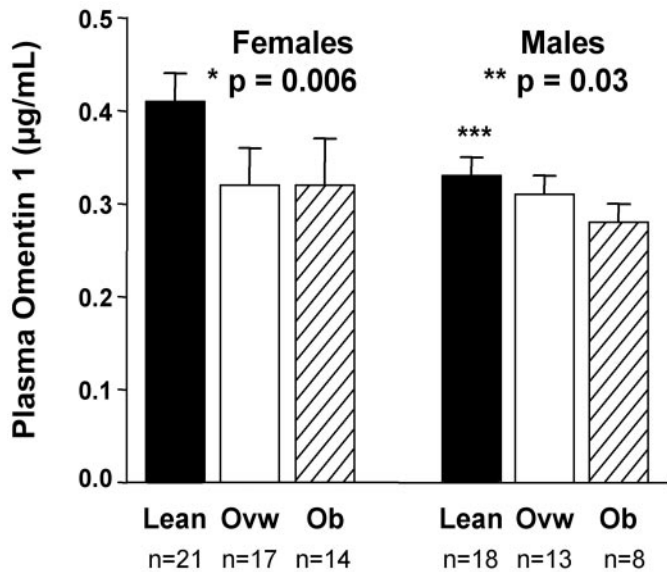


FIG. 3. Circulating omentin 1 levels are decreased with obesity. Data are means \pm SE. Lean ($n = 21$), overweight (ovw, $n = 17$), and obese (ob, $n = 14$) values for women were 0.41 ± 0.03 , 0.32 ± 0.04 , and 0.32 ± 0.05 , respectively. Lean ($n = 18$), overweight (ovw, $n = 13$), and obese (ob, $n = 8$) values for men were 0.33 ± 0.02 , 0.30 ± 0.03 and 0.29 ± 0.03 , respectively. P values for the sex-stratified analysis between the three groups in men and women ($*P = 0.03$ and $**P = 0.006$, respectively) were determined by variance components analysis adjusted for age, sex, and family structure. Plasma omentin 1 levels were higher in lean women than in lean men ($***P = 0.03$).

Omentin gene expression correlates negatively with BMI. Omentin 1 and omentin 2 mRNA levels (copy numbers) were measured in visceral fat from surgical subjects by real-time quantitative RT-PCR using standard curves generated with omentin 1-containing (11) and omentin 2-containing (pcDNA3) plasmids. Using Spearman regression analysis adjusted for sex and age, significant negative correlations between BMI and omentin 1 ($n = 20$, $r_s = -0.62$, $P = 0.006$) and omentin 2 mRNA levels ($n = 20$, $r_s = -0.73$, $P = 0.0006$) were found (Fig. 5A and B). Additionally, when comparing omentin 1 and omentin 2 gene expression, a highly significant positive correlation was observed ($n = 20$, $r_s = 0.76$, $P = 0.0002$; Fig. 5C). Although omentin 1 and 2 gene expressions are highly correlated in visceral adipose tissue, the mRNA level of omentin 1 is ~ 50 times greater than omentin 2. In

light of the small number of men (2 of 20), the same analysis adjusted for age was performed only with women and revealed essentially the same results. Both omentin 1 and 2 gene expressions correlated negatively with BMI ($n = 18$, $r_s = -0.62$, $P = 0.01$ and $n = 18$, $r_s = -0.73$, $P = 0.001$, respectively). In addition, omentin 1 and 2 correlate positively with each other ($n = 18$, $r_s = 0.75$, $P = 0.0005$).

Omentin 1 gene expression correlates positively with omentin 1 plasma levels. To determine whether a linear relationship exists between omentin gene expression and plasma omentin levels, visceral adipose gene expression of omentin 1 was compared with plasma omentin 1 levels from a subgroup of the surgical subjects. Omentin 1 mRNA levels were positively correlated with omentin 1 plasma levels ($n = 14$, $r_s = 0.60$, $P = 0.02$; Fig. 6), thus illustrating a positive relationship between omentin 1 gene regulation and omentin 1 levels in the circulation.

DISCUSSION

Visceral obesity is highly associated with risks for type 2 diabetes, cardiovascular disease, hypertension, and hyperlipidemia (1–4). Expression of adipose-derived factors such as leptin (27), plasminogen activator inhibitor 1 (28), and adiponectin (29,30) are modulated by obesity. Therefore, our study of circulating omentin levels and omentin gene expression was undertaken to better understand the regulation and role of omentin, a new visceral fat depot-specific adipokine, in obesity.

Two-dimensional gel electrophoresis followed by Western blotting was conducted to determine which omentin isoforms or other cross-reactive species contributed to the immunoreactivity in the one-dimensional Western assay that was subsequently used to quantitate plasma omentin levels. Based on the cross-reactivity of the 3G1B3 antibody and the differing pIs between omentin 1 and 2, we demonstrated that omentin 1 is the major circulating form in plasma. Other apparent cross-reactive species were not observed in the same molecular weight range of the omentins, thus validating the specificity of the monoclonal antibody 3G1B3 for omentin in human plasma by two-dimensional gel electrophoresis and its utility in the quantitative Western blotting assay.

As previously reported (11), visceral fat is the tissue exhibiting the highest level of omentin 1 expression. However, compared with omentin 1, omentin 2 is expressed in considerably lower levels in visceral fat and in

TABLE 2

Correlations of metabolic syndrome-related traits with plasma omentin 1 levels in 91 Amish subjects

Covariate	Age and sex		Age, sex, and BMI		Age, sex, and waist	
	r	P	r	P	r	P
BMI (kg/m^2)	-0.34	0.0007	—	—	-0.09	0.4
Adiponectin ($\mu\text{g}/\text{ml}$)	0.29	0.005	0.16	0.1	0.15	0.1
Ln leptin (ng/ml)	-0.41	<0.0001	-0.25	0.02	-0.24	0.02
Ln HOMA index	-0.33	0.003	-0.18	0.09	-0.14	0.2
HDL cholesterol (mmol/l)	0.31	0.003	0.15	0.2	0.20	0.06
Waist circumference (cm)	-0.35	0.0005	-0.12	0.2	—	—
LDL cholesterol (mmol/l)	-0.16	0.1	-0.11	0.3	-0.10	0.4
Ln triglycerides (mmol/l)	-0.19	0.07	-0.09	0.4	-0.12	0.3
Total cholesterol (mmol/l)	-0.04	0.7	-0.03	0.8	0.00	0.9
Ln insulin ($\mu\text{U}/\text{ml}$)	-0.31	0.003	-0.16	0.1	-0.13	0.2
Glucose (mg/dl)	-0.09	0.4	0.02	0.8	0.00	1.0
Systolic blood pressure (mmHg)	-0.20	0.05	-0.05	0.7	-0.03	0.8
Diastolic blood pressure (mmHg)	-0.03	0.8	0.09	0.4	0.15	0.1

All analyses incorporate residual correlations between family members using variance components analysis as implemented in SOLAR. Ln, natural log transformed.

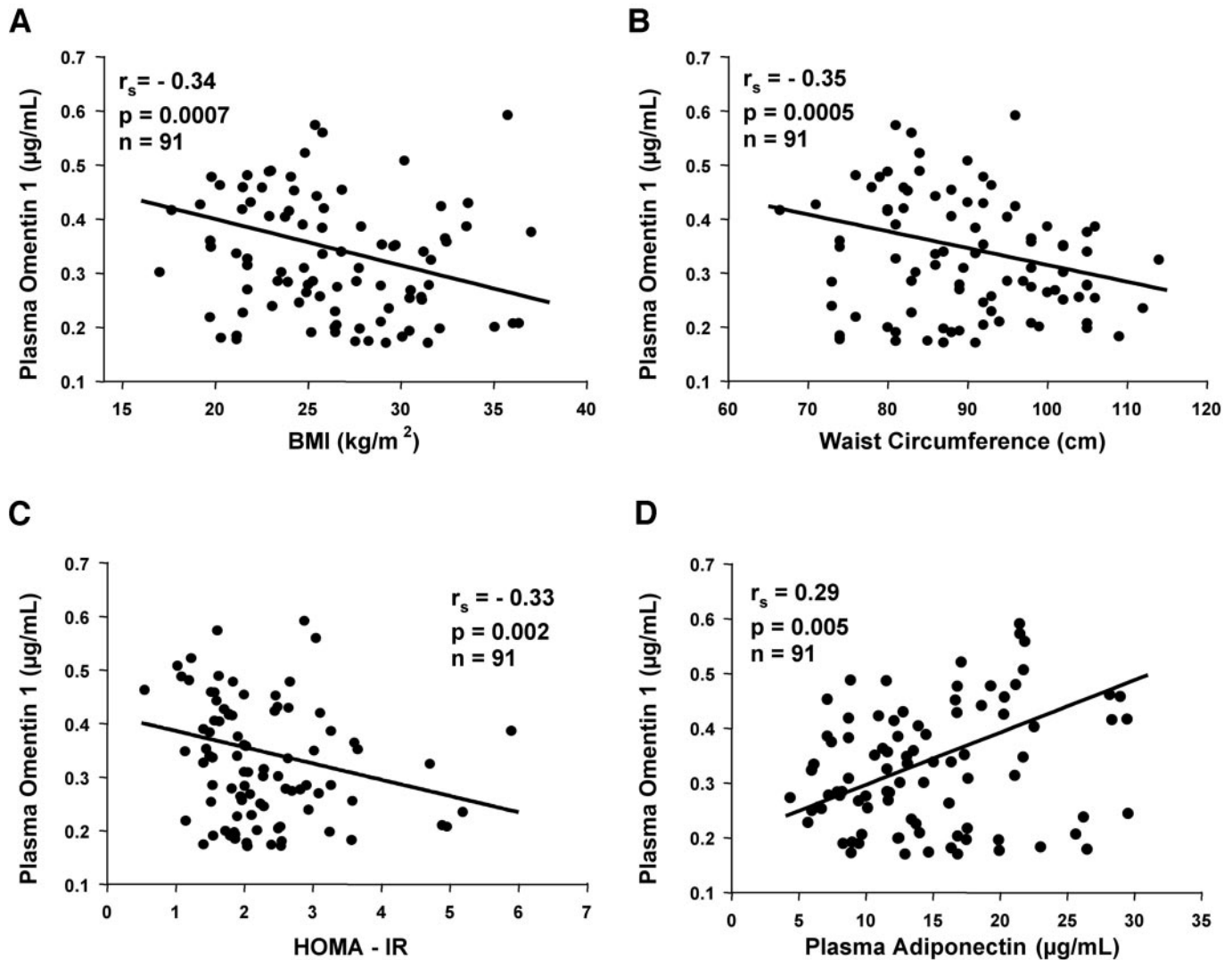


FIG. 4. Plasma omentin 1 levels correlations with obesity and insulin resistance markers. Significant correlation coefficients based on variance components analysis adjusted for sex, age, and family structure were found between plasma omentin 1 levels with BMI ($n = 91$, $r = -0.34$, $P = 0.0007$) (A); waist circumference ($n = 91$, $r = -0.35$, $P = 0.0005$) (B); Ln HOMA index ($n = 91$, $r = -0.33$, $P = 0.002$) (C); and plasma adiponectin levels ($n = 91$, $r = 0.29$, $P = 0.005$) (D).

higher levels in the intestine (data not shown). Release of omentin 2 into the intestinal lumen and the difference in visceral fat expression of the two isoforms may explain why omentin 2 was not detected in human plasma. Although omentin 1 and omentin 2 exhibit a different pattern of tissue expression, they may share some similar regulation by obesity as evidenced by the negative correlations between the gene expression of both isoforms and BMI and the positive correlation with each other in visceral adipose tissue.

Plasma omentin 1 levels were measured in a well-characterized genetically homogeneous population of Old Order Amish (24). As expected for a visceral depot-specific adipocytokine that positively affects insulin sensitivity, higher plasma omentin 1 levels were observed in lean versus obese and overweight subjects independent of age, sex, and family structure. Further evidence to suggest that omentin 1 may be involved in some aspect of insulin sensitivity is the negative correlation between omentin 1 plasma levels and measurements of insulin resistance (HOMA). Moreover, negative correlations between plasma omentin 1 levels and BMI, waist circumference, and leptin

values were observed. These data suggest that some aspect of obesity negatively regulates omentin expression and release into the circulation.

It is interesting to note that this pattern of results for omentin 1 is similar to adiponectin, an insulin sensitizer and cardio-protective adipokine (31–36). In fact, circulating total plasma adiponectin levels were positively correlated with plasma omentin 1 values in this study. The inverse relationship between obesity and both omentin and adiponectin may suggest similar regulation. It is also possible that regulation of omentin may be dependent on adiponectin or vice versa. Visfatin, the proposed insulin-mimetic adipokine, also shows negative correlations between plasma levels and BMI (37). However, despite similar relationships with BMI for omentin 1, adiponectin, and visfatin, omentin solely exhibits visceral fat depot-specific expression.

A difference in plasma omentin 1 levels between lean women and men was observed. This difference was not highly significant probably because of the small sample size. Therefore, future studies will be required to address

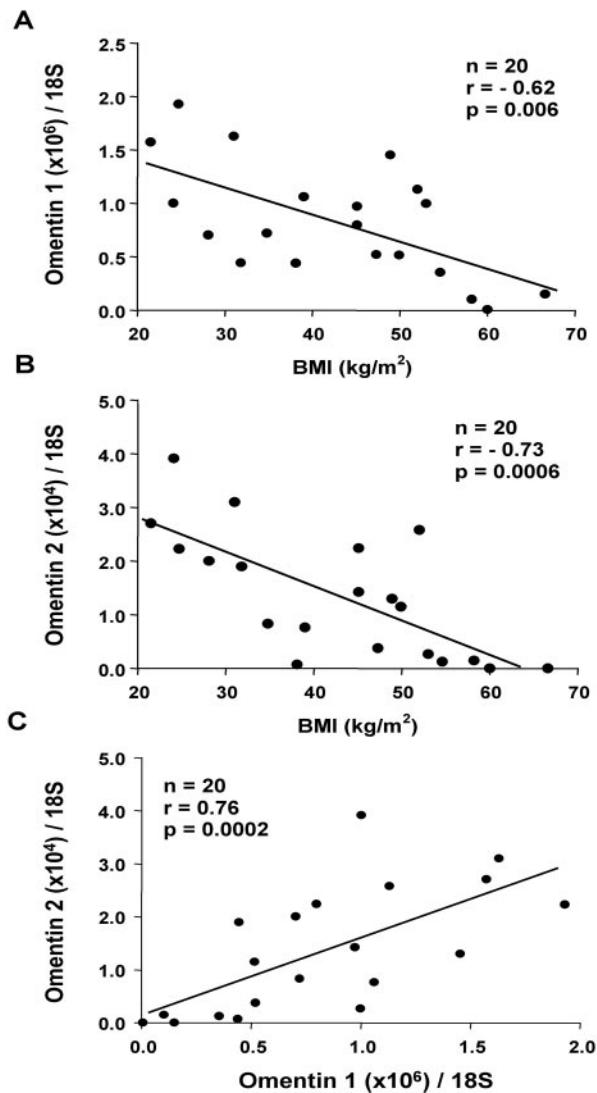


FIG. 5. Visceral adipose omentin 1 and 2 gene expression levels are decreased with BMI. Visceral adipose omentin 1 (A) and omentin 2 (B) mRNA copies-to-18S RNA ratio, measured by quantitative RT-PCR, were negatively correlated with BMI ($n = 20$, $r_s = -0.62$, $P = 0.006$; $n = 20$, $r_s = -0.73$, $P = 0.0006$, respectively; Spearman correlations adjusted for sex and age). C: There was a significant positive correlation between omentin 1 and 2 gene expression levels ($n = 20$, $r_s = 0.76$, $P = 0.0002$; Spearman correlation adjusted for sex and age).

this apparently divergent regulation of omentin between men and women.

When plasma omentin is adjusted for BMI or waist circumference in the covariate analysis, there is a loss of significance with most omentin-associated traits. Plasma leptin was still significant after adjustment although reduced in magnitude. These results reinforce the concept that plasma omentin 1 levels are highly regulated by obesity and will lose significant associations with obesity-dependent variables when adjusted by BMI or waist circumference. The modestly significant residual correlation between leptin and omentin independent of BMI or waist circumference may be indicative of a regulatory relationship between these two adipokines outside of the effects of adiposity.

To extend the relationship between plasma omentin and obesity to omentin gene expression, omentin 1 and 2 mRNA levels were measured in visceral adipose tissue from surgical subjects. Similar to plasma omentin levels,

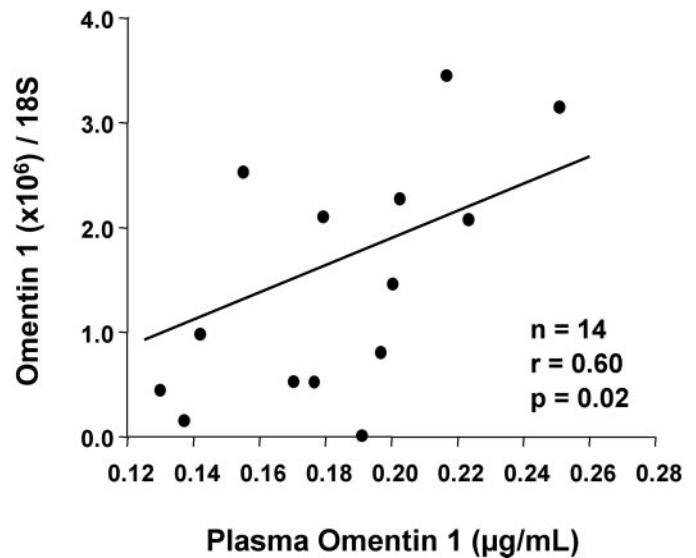


FIG. 6. Omentin 1 gene expression was positively correlated with human plasma omentin 1 concentrations. Visceral omentin 1 mRNA copies-to-18S RNA ratio, measured by quantitative RT-PCR, was positively correlated with human plasma omentin 1 levels ($n = 14$, $r_s = 0.60$, $P = 0.02$; Spearman correlation).

visceral adipose omentin gene expression for both isoforms, omentin 1 and omentin 2, is lower in the obese state than in the lean state, as evidenced by negative correlations between gene expression and BMI. In fact, mRNA levels for omentin 1 and 2 are highly correlated with each other in omental adipose tissue. In contrast with omentin and adiponectin (38), which show obesity-dependent decreases in gene expression, visfatin was previously shown to have obesity-dependent increases in visceral fat gene expression (37,39). Correlation between omentin 1 mRNA and plasma omentin 1 levels was also observed, suggesting that the regulation of omentin 1 gene expression in visceral fat can predict the circulating levels of omentin 1.

Although the data clearly support regulation of omentin by obesity, omentin may also be regulated by inflammation. Other studies have shown that omentin 1 (also known as intelectin 1) expression is altered in inflammatory states (12,40). Obesity itself is associated with low levels of chronic inflammation, which may contribute to the regulation in the role of omentin in human physiology (41-43). Consequently, weight loss and different inflammatory states could be modulators of omentin expression and function.

In summary, we find that plasma omentin 1 and both omentin 1 and 2 mRNAs are inversely related to obesity. Women have higher levels of circulating omentin 1 and a larger range of variation with BMI. Plasma omentin 1 correlates negatively with BMI, leptin, waist circumference, fasting insulin, and HOMA and positively with adiponectin and HDL. Association with these metabolic indexes suggests that higher omentin levels may be seen as a marker for leanness or as a positive factor that opposes the obese state and its pathophysiological consequences.

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