Omentin-1, a Novel Adipokine, Is Decreased in Overweight Insulin-Resistant Women With Polycystic Ovary Syndrome

Ex Vivo and In Vivo Regulation of Omentin-1 by Insulin and Glucose

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OBJECTIVE—Polycystic ovary syndrome (PCOS) is associated with insulin resistance and obesity. Recent studies have shown that plasma omentin-1 levels decrease with obesity. Currently, no data exist on the relative expression and regulation of omentin-1 in adipose tissue of women with PCOS. The objective of this study was to assess mRNA and protein levels of omentin-1, including circulating omentin-1, in omental adipose tissue of women with PCOS and matched control subjects. Ex vivo and in vivo regulation of adipose tissue omentin-1 was also studied.

RESEARCH DESIGN AND METHODS–Real-time RT-PCR and Western blotting were used to assess mRNA and protein expression of omentin-1. Plasma omentin-1 was measured by enzyme-linked immunosorbent assay. The effects of D-glucose, insulin, and gonadal and adrenal steroids on adipose tissue omentin-1 were analyzed ex vivo. The in vivo effects of insulin (hyperinsulinemia) on omentin-1 levels were also assessed by a prolonged insulin-glucose infusion.

RESULTS—In addition to decreased plasma omentin-1 levels in women with PCOS ($P < 0.05$), compared with control subjects, there was significantly lower levels of omentin-1 mRNA ($P < 0.01$) and protein ($P < 0.05$) in omental adipose tissue of women with PCOS ($P < 0.01$). Furthermore, in omental adipose tissue explants, insulin and glucose significantly dose-dependently decreased omentin-1 mRNA expression, protein levels, and secretion into conditioned media ($P < 0.05$, $P < 0.01$). Also, hyperinsulinemic induction in healthy subjects significantly reduced plasma omentin-1 levels ($P < 0.01$).

CONCLUSIONS—Our novel findings reveal that omentin-1 is downregulated by insulin and glucose. These may, in part, explain the decreased omentin-1 levels observed in our overweight women with PCOS. ***Diabetes*** **57:801–808, 2008**

Polycystic ovary syndrome (PCOS), a common endocrinopathy affecting 5–10% of women in the reproductive age, is characterized by irregular menses and hyperandrogenism and is associated with insulin resistance and pancreatic β-cell dysfunction, impaired glucose tolerance, type 2 diabetes, dyslipidemia, and visceral obesity (1,2). The consequent hyperinsulinemia is more prevalent in lean and obese women with PCOS compared with age- and weight-matched normal women (3).

The metabolic syndrome is associated with excessive accumulation of central body fat. As well as its role in energy storage, adipose tissue produces several hormones and cytokines termed adipokines that have widespread effects on carbohydrate and lipid metabolism. They appear to play an important role in the pathogenesis of insulin resistance, diabetes, and atherosclerosis (4). Furthermore, it is apparent that accumulation of visceral adipose tissue poses a greater cardiometabolic risk than subcutaneous adipose tissue (5), as removal of visceral rather than subcutaneous adipose tissue has been shown to improve insulin sensitivity (6). Moreover, differences in gene expression of adipocyte-secreted molecules (adipokines) suggest that there are inherent adipose tissue depot–specific differences in the endocrine function of adipose tissue. In relation to this, we have published data (7) on the increased levels of visfatin in women with PCOS, visfatin being a recently described adipokine mainly formed in human visceral adipose tissue that has insulin-mimetic effects by binding and activating the insulin receptor in a manner distinct from that of insulin.

More recently, expressed sequence tag analyses from a human omental adipose tissue cDNA library led to the identification of a novel adipokine, named omentin-1, preferentially produced by visceral adipose tissue compared with subcutaneous adipose tissue. Furthermore, in vitro experiments revealed that treatment with recombinant omentin-1 enhances insulin-stimulated glucose uptake in human subcutaneous and omental adipocytes. Also, omentin-1 was shown to trigger Akt signaling in both the absence and presence of insulin (9,10). Furthermore,

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Received for publication 17 July 2007 and accepted in revised form 23 December 2007.

Published ahead of print at http://diabetes.diabetesjournals.org on 3 January 2008. DOI: 10.2337/db07-0990.

Additional information for this article can be found in an online appendix at http://dx.doi.org/10.2337/db07-0990.

DHEA-S, dehydroxyepiandrostenedione-sulfate; ELISA, enzyme-linked immunosorbent assay; HOMA, homeostasis model assessment; PCOS, polycystic ovary syndrome; WHR, waist-to-hip ratio.

TABLE 1
Clinical, hormonal, and metabolic features of women with PCOS and control subjects

Variable	PCOS	Control subjects	Significance
n	10	10	
Age (year)	32.2 ± 5.8	32.7 ± 4.5	NS
BMI (kg/m^2)	29.9 ± 2.3	29.2 ± 2.6	NS
WHR	0.87 ± 0.15	0.85 ± 0.16	NS
Glucose (mmol/l)	5.2 ± 0.7	4.6 ± 0.5	$P < 0.05$
Insulin (pmol/l)	90.9 ± 38.8	52.4 ± 15.0	$P < 0.05$
HOMA	3.5 ± 1.8	1.8 ± 0.6	$P < 0.01$
Cholesterol (mmol/l)	5.0 ± 0.8	5.2 ± 0.8	NS
Fasting triglycerides (mmol/l)	2.2 ± 1.2	1.0 ± 0.4	$P < 0.01$
Leutinizing hormone (IU/l)	8.6 ± 4.3	6.0 ± 4.2	NS
Follicle-stimulating hormone (IU/l)	6.3 ± 1.6	6.4 ± 2.7	NS
Prolactin (mU/l)	357.9 ± 87.9	298.0 ± 85.8	NS
E_2 (pmol/l)	365.4 ± 101.0	207.5 ± 67.8	$P < 0.01$
Progesterone (nmol/l)	1.9 ± 0.6	2.1 ± 0.4	NS
17-OH-P (nmol/l)	2.5 ± 0.7	1.9 ± 0.6	NS
Testosterone (nmol/l)	3.9 ± 1.1	2.4 ± 0.5	$P < 0.01$
Androstenedione (nmol/l)	13.0 ± 3.4	8.6 ± 2.3	$P < 0.01$
DHEA-S (μmol/l)	6.0 ± 1.3	4.7 ± 1.1	NS
Sex hormone–binding globulin (nmol/l)	31.6 ± 6.9	53.1 ± 16.6	$P < 0.01$
Free androgen index	12.3 ± 5.3	4.5 ± 8.1	$P < 0.01$
Omentin-1 (ng/ml)	255.8 ± 78.2	348.0 ± 112.6	$P < 0.05$

Data are means ± SD. Group comparison by Mann-Whitney *U* test. FAI = T (nmol/l)/sex hormone–binding globulin (nmol/l) × 100. NS, not significant.

omentin plasma levels and omentin gene expression in visceral adipose are decreased in obesity (10).

As PCOS is a prodiabetic state with a higher prevalence of obesity (1,2), we measured plasma omentin-1 levels and studied the mRNA expression and protein levels of omentin-1 in both subcutaneous and omental adipose tissue depots in these women and compared them with age-, BMI-, and waist-to-hip ratio (WHR)-matched control subjects. Because PCOS is a state of hyperinsulinemia with altered gonadal and adrenal steroid levels, we also assessed the effects of glucose with and without insulin and steroid hormones on omentin-1 secretion into conditioned media as well as mRNA expression and protein levels from human omentaladipose tissue explants. Finally, we studied the effects of hyperinsulinemia on plasma omentin-1 levels via a prolonged insulin-glucose infusion in healthy subjects.

RESEARCH DESIGN AND METHODS

Study 1. All PCOS patients met all three criteria of the revised 2003 Rotterdam European Society of Human Reproduction/American Society for Reproductive Medicine PCOS Consensus Workshop Group diagnostic criteria. The three criteria are *1*) oligo- and/or anovulation, *2*) clinical and/or biochemical signs of hyperandrogenism, and *3*) polycystic ovaries (11). Furthermore, all subjects in the control arm had normal findings on pelvic ultrasound scans, regular periods, and no hirsutism/acne. The control group had no discernible cause for infertility (unexplained infertility). No women were amenorrheic. All subjects that were studied did not have endometriosis. Exclusion criteria for the study included age >40 years, known cardiovascular disease, thyroid disease, neoplasms, current smoking, diabetes, hypertension (blood pressure >140/90 mmHg), and renal impairment (serum creatinine >120 μmol/l). None of the women were on any medications for at least 6 months before the study, including oral contraceptives, glucocorticoids, ovulation induction agents, antidiabetic and antiobesity drugs, estrogenic, or antiandrogenic or antihypertensive medication. Also, the presence of other endocrinopathies were ruled out by measuring basal serum 17-hydroxyprogesterone, prolactin, and 0800–0900 h cortisol after 1.0-mg (2300 h) overnight dexamethasone suppression (values <30 nmol/l were considered to rule out Cushing's syndrome). All subjects suppressed cortisol <30nmol/l.

After an overnight fast, blood samples and subcutaneous and omentaladipose tissue were obtained (0800–1000 h) from adult female patients undergoing elective surgery for infertility investigation. Subjects were initially seen at the infertility clinic and then scheduled for laparoscopy to assess Fallopian tube(s) patency. All subjects underwent anthropometric measurements (i.e., weight, height, and WHR). A total of 51 subjects were recruited consecutively from the infertility clinic in accordance with the inclusion/exclusion criteria (PCOS: $n = 13$; control subjects: $n = 38$). Of 13 PCOS subjects recruited, 3 withdrew before the study could be completed. In the control group, four subjects did not complete the study. From the remaining 34 control subjects, 10 control subjects matched for age, BMI, and WHR were included in the final analysis (Table 1). Subcutaneous biopsies were obtained from the same site (i.e., from a 3-cm horizontal midline incision ~3 cm above the symphysis pubis). All samples were obtained during the early follicular phase (days 2–4 from the first day of the spontaneous bleeding episode). Plasma was immediately aliquoted on ice and stored at −80°C. The same fat pad was divided equally into two halves. Each half was either immediately frozen in liquid nitrogen and stored at −80°C or placed into a sterile container containing medium 199 (Sigma-Aldrich, Gillingham, U.K.) for primary adipose tissue culture. All patients underwent anthropometric measurements (i.e., weight, height, and WHR). The local research ethics committee approved the study, and all patients involved gave their informed consent in accordance with the guidelines in the 2000 Declaration of Helsinki.

Study 2. We measured omentin-1 in six healthy subjects (age [means ± SD] 26.5 ± 8 years, BMI 23.2 ± 2.5 kg/m^2). None of the subjects were on any medications for at least 6 months before the study. To account for the possibility of diurnal variation in omentin-1 levels, we initially obtained a daily control curve by measuring fasting omentin-1 levels at 30-min intervals from 0800 to 1000 h. Subsequently, nonfasting omentin-1 levels were measured at 2-hourly intervals until 2400 h and then at 0400 h ,as well as at 30-min intervals from 0800 to 1000 h on day 2. On the following day the same subjects were subjected to a prolonged insulin-glucose infusion for 26 h beginning at 0800 h. Insulin (Human Actrapid) was administered intravenously as a priming dose of 0.04 units/kg, followed by continuous infusion of 0.5 mU/kg/min. By choosing this rate of insulin infusion, we expected to achieve hyperinsulinemia with an approximate four- to sixfold elevation of basal insulinemia, such a rise being similar to peak values observed during an oral glucose tolerance test (12). Fasting blood samples were drawn at 30-min intervals between 0800 and 1000 h on day 1 and day 2 of the prolonged insulin-glucose infusion (the first and the last 2 h of the infusion). Intermediate blood samples (nonfasted) were taken at 2-h intervals until 2400 h and then at 0400 h on day 2. Preprandial glucose levels were maintained between 4.0 and 6.0 mmol/l.

Biochemical and hormonal analysis. Assays for glucose, insulin, luteinizing hormone, follicular stimulating hormone, 17β-estradiol (E_2), progesterone, testosterone, androstenedione, dehydroxyepiandrostenedione-sulfate (DHEA-S), and sex hormone–binding globulin were performed using an automated

analyzer (Abbott Architect; Abbott Laboratories, Abbott Park, IL). The estimate of insulin resistance by homeostasis model assessment (HOMA) score was calculated as $I_o \times G_o/22.5$, where I_o is fasting insulin and G_o is fasting glucose, as described by Matthews et al. (13). Omentin levels in plasma and conditioned media from human omental adipose tissue explants were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) (ELISA kit; Axxora, Nottingham, U.K.), according to manufacturer's protocol, with an intra-assay coefficient of variation of <6%.

Primary explant culture. Adipose tissue organ explants were cultured using a protocol that was a modification of the method described by Fried and Moustaid-Moussa (14). Briefly, 1–3 g of adipose tissue was minced into 5- to 10-mg (~1 mm^3) fragments, washed with a 230-μm mesh (filter no. 60; Sigma-Aldrich) and rinsed with sterile PBS warmed to 37°C. Samples were then transferred to six-well plates (~50 mg/well) containing 3 ml of media 199 (Life Technologies–BRL, Paisley, U.K.) supplemented with 50 μg/ml gentamicin and 1% fetal bovine serum (containing insulin at a concentration of 10^{-14} mol/l) and cultured for 24 h with or without the addition of testosterone, 17β-estradiol, androstenedione, DHEA-S, insulin, or D-glucose in a 37°C incubator under an atmosphere of 5% CO_2/95% air.

Total RNA extraction and cDNA synthesis. Total RNA was extracted from whole adipose tissue samples and isolated adipocytes using Qiagen RNeasy Lipid Tissue Mini Kit according to the manufacturer's guidelines (Qiagen, Sussex, U.K.). The purity of the extracted RNA was measured by a NanoDrop spectrophotometer. A set concentration of RNA was reverse transcribed into cDNA, by using Moloney murine leukemia virus reverse transcriptase (Fermentas, York, U.K.) and random hexamers (Promega, Southampton, U.K.) as primers.

RT-PCR. Quantitative PCR of omentin-1 was performed on a Roche Light Cycler system (Roche Molecular Biochemicals, Mannheim, Germany). PCR were carried out in a reaction mixture consisting of 5.0 μl reaction buffer and 2.0 mmol/l $MgCl_2$ (Biogene, Kimbolton, U.K.), 1.0 μl of each primer (10 ng/μl), 2.5 μl of cDNA, and 0.5 μl of Light Cycler DNA Master SYBR Green I (Roche). Protocol conditions consisted of denaturation of 95°C for 15 s, followed by 40 cycles of 94°C for 1 s, 58°C for 10 s, and 72°C for 12 s, followed by melting-curve analysis. For analysis, quantitative amounts of genes of interest were standardized against the housekeeping gene β-actin. The RNA levels were expressed as a ratio, using the δ-δ method for comparing relative expression results between treatments in real-time PCR (15). The sequences of the sense and antisense primers used were omentin-1 (199 bp) 5′-AACAGCTCCCTGCTGAGGTA-3′ and 5′-GCTGGCCATAGGGTGAGTAA-3′ and β-actin (216bp) 5′-AAGAGAGGCATCCTCACCCT-3′ and 5′-TACATGGCT GGGGTCTTGAA-3′.

Ten microliters of the reaction mixture(s) were subsequently electrophoresed on a 1% agarose gel and visualized by ethidium bromide, using a 1-kb DNA ladder (Life Technologies–BRL) in order to estimate the band sizes. As a negative control for all the reactions, preparations lacking RNA or reverse transcriptase were used in place of cDNA. RNAs was assayed from three independent biological replicates.

Western blotting. Protein lysates were prepared by homogenizing adipose tissue in radioimmunoprecipitation lysis buffer (Upstate, Lake Placid, NY), according to manufacturer's instructions. Protein samples (30 μg/lane) containing SDS sample buffer (5 mol/l urea, 0.17 mol/l SDS, 0.4 mol/l dithiothreitol, and 50 mmol/l Tris-HCl, pH 8.0) were subjected to SDS-PAGE (10% resolving gel) and transferred to polyvinylidene difluoride membranes. The polyvinylidene difluoride membranes were incubated with primary mouse anti-human antibody for omentin-1 (Axxora, Nottingham, U.K.) (1:1,000 dilution) or primary rabbit anti-human antibody for β-actin (Cell Signaling Technology, Beverly, MA) (1:1,000 dilution) overnight at 4°C. The membranes were washed thoroughly for 60 min with TBS-0.1% Tween before incubation with the secondary anti-rabbit horseradish peroxidase–conjugated Ig (Dako, Ely, Cambridgeshire, U.K.) (1:2,000) for 1 h at room temperature. Antibody complexes were visualized using electrochemiluminescence (ECL+; Amersham, Little Chalfont, Buckinghamshire, U.K.). Human omentin-1 peptide (Axxora) was used as the positive control and water as the negative control (data not shown).

Statistics. Nonparametric tests were used. Data are presented as means ± SE, unless indicated otherwise. Differences between two groups were assessed using the Mann-Whitney *U* test. Data involving more than two groups were assessed by Friedman's ANOVA with Dunn's test for post hoc analysis. For Western immunoblotting experiments, the densities were measured using a scanning densitometer coupled to scanning software Scion Image (Scion, Frederick, MD). Spearman rank correlation was used for calculation of associations between variables. $P < 0.05$ was considered significant.

RESULTS

Study 1: demographic data. Table 1 shows the anthropometric, biochemical, and hormonal data in women with PCOS and control women. Glucose, insulin, HOMA, triglycerides, 17β-estradiol, testosterone, and androstenedione levels and free androgen index were significantly higher, whereas sex hormone–binding globulin was significantly lower, in women with PCOS. ELISA analysis of plasma omentin-1 levels revealed that patients with PCOS had significantly lower levels when compared with control subjects (255.8 ± 78.2 vs. 348.0 ± 112.6 ng/ml; $P < 0.05$) (Table 1). Serum progesterone levels in all women confirmed follicular phase of the menstrual cycle.

mRNA expression and protein levels of omentin-1 in normal and women with PCOS. We detected omentin-1 mRNA in omental adipose tissue and subsequent sequencing of the PCR products confirmed gene identity. However, omentin-1 mRNA expression was barely detectable in all subcutaneous adipose tissue samples (data not shown). Real-time RT-PCR analysis corrected over β-actin showed a significant decrease of omentin-1 in omental ($P < 0.01$) adipose tissue of women with PCOS when compared with normal control subjects (Fig. 1*A*). The changes noted at the mRNA level were also reflected at the protein level in women with PCOS (i.e., significantly lower omentin-1 levels in omental adipose tissue of women with PCOS) (Fig. 1*B*) (*$P < 0.05$).

Dose-dependent effects of D-glucose and insulin on omentin-1 net protein production and secretion into conditioned media from control human omental adipose tissue explants. Omentin-1 net protein production was significantly decreased dose dependently by D-glucose (20 and 40 mmol/l) from control human omental adipose tissue explants (Fig. 2*A*) ($P < 0.05$ and $P < 0.01$, respectively). Similar observations were noted with respect to secretion of omentin-1 into conditioned media in corresponding adipose tissue explants (Fig. 2*B*) ($P < 0.05$ and $P < 0.01$, respectively).

Further, omentin-1 net protein production and secretion into the conditioned media was significantly decreased dose dependently by insulin in the presence of 5 mol/l D-glucose from control human omental adipose tissue explants (Fig. 3*A* and *B*) ($P < 0.05$ and $P < 0.01$, respectively). Of note, a similar pattern of omentin-1 regulation by insulin was observed at higher concentrations of D-glucose (30 mmol/l) (data not shown).

Effects of testosterone, 17β-estradiol, androstenedione, and DHEA-S on omentin-1 levels in control human omental adipose tissue explants. Given that women with PCOS have elevated gonadal and adrenal steroids, we investigated the effects of these steroids ex vivo. Interestingly, there was no significant difference noted in omentin-1 net protein production with testosterone, 17β-estradiol, androstenedione, or DHEA-S treatments ($P > 0.05$) (data not shown).

Association of omentin-1 with covariates. Plasma omentin-1, omental adipose tissue omentin-1 mRNA expression, and protein levels were negatively associated with BMI, WHR, glucose, HOMA, and 17β-estradiol ($P < 0.01$). Moreover, similar findings were noted when the groups were analyzed individually (Table 2 of the online appendix, available at http://dx.doi.org/10.2337/db07-0990).

Given the observation that glucose and insulin decreased omentin-1 net protein production and secretion

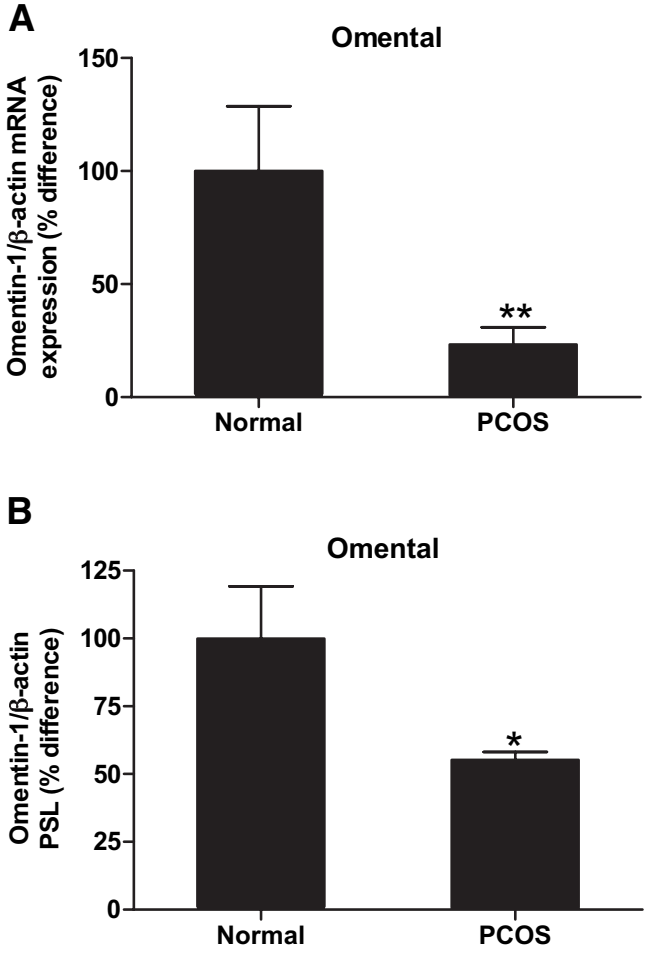

FIG. 1. *A*: **Omentin-1 mRNA expression relative to β-actin is significantly decreased in human omental adipose tissue depots when comparing all women with PCOS to all normal control subjects, using real-time RT-PCR. Data are means ± SE. Group comparison by Mann-Whitney *U* test. **$P < 0.01$. *B*: Densitometric analysis of omentin-1 immune complexes having normalized to β-actin, respectively, revealed that protein levels of omentin-1 is significantly decreased in human omental adipose tissue depots when comparing all women with PCOS to all normal control subjects. Data are means ± SE. Group comparison by Mann-Whitney *U* test. *$P < 0.05$. PSL, phospho-stimulated light units.**

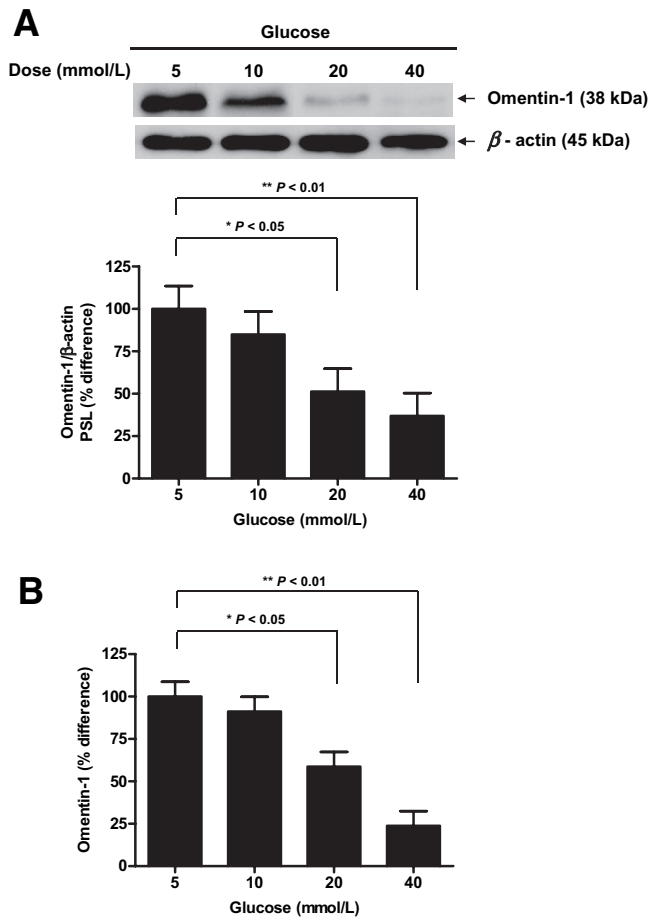

FIG. 2. *A*: **Dose-dependent effects of D-glucose (5, 10, 20, and 40 mmol/l) on omentin-1 net protein production in control human omental adipose tissue explants at 24 h was assessed by Western blotting. Western blot analysis of protein extracts from omental adipose tissues demonstrate that the antibody against omentin-1 and the antibody against β-actin recognized bands with apparent molecular weights of 38 and 45 kDa, respectively (*A*, *inserts*). Densitometric analysis of omentin-1 immune complexes having normalized to β-actin, respectively, revealed that protein levels of omentin-1 were significantly decreased by D-glucose (20 and 40 mmol/l) in control human omental adipose tissue explants. Data are means ± SE of six experiments. Each experiment was carried out with six different samples from six different subjects in three replicates. Group comparison by Friedman's ANOVA and post hoc Dunn's test. *$P < 0.05$; **$P < 0.01$. *B*: Dose-dependent effects of D-glucose on omentin-1 secretion into conditioned media from control human omental adipose tissue explants at 24 h were measured by ELISA. Omentin-1 secretion was significantly decreased by D-glucose (20 and 40 mmol/l) from human omental adipose tissue explants. Data are means ± SE of six experiments. Each experiment was carried out with six different samples from six different subjects in three replicates. Group comparison by Friedman's ANOVA and post hoc Dunn's test. *$P < 0.05$; **$P < 0.01$.**

into condition media (Figs. 2*A* and *B* and 3*A* and *B*), Spearman rank analyses demonstrated that plasma omentin-1 significantly negatively correlated with HOMA (Fig. 4*A*) ($R = -0.56$; $P < 0.05$) but also omental adipose tissue mRNA expression (Fig. 4*B*) ($R = -0.58$; $P < 0.01$) and omental adipose tissue protein levels (Fig. 4*C*) ($R = -0.60$; $P < 0.01$). Furthermore, omental adipose tissue mRNA expression and protein levels significantly positively correlated with plasma omentin-1 (Fig. 4*D* and *E*) ($R = 0.96$, $P < 0.01$; $R = 0.82$, $P < 0.01$, respectively). Once again, these findings were consistent when the groups were analyzed individually (Table 2) (see online appendix).

Study 2: effects of a prolonged insulin-glucose infusion on plasma omentin-1 levels. In study 2, insulin infusion resulted in elevation of fasting insulinemia from 78.1 ± 12 to 294.6 ± 31pmol/l. Insulin levels remained elevated until the end of the prolonged insulin-glucose infusion (366.0 ± 37 pmol/l), thus achieving our objective of an approximate four- to sixfold elevation of fasting insulinemia. With respect to omentin-1 levels, there was no overall statistically significant difference between the starting levels for the control and infusion periods. Omentin-1 levels remained essentially unaltered throughout the control day from 182 ± 17 ng/ml at 0800 h to 205 ± 126 ng/ml at 1000 h the next day (Fig. 5*A* and *B*) ($P > 0.05$).

There was, however, a potent repressive effect of insulin on omentin-1 levels over 26 h of insulin infusion: from 165 ± 44 ng/ml at 0800 h to 37 ± 14 ng/ml at 1000 h the following day (Fig. 5*A* and *B*) ($P < 0.01$). Of interest, the initial decline in circulating omentin-1 level was relatively acute achieving a nadir at 4 h.

DISCUSSION

Omentin-1 mRNA expression, a new adipokine (10), has recently been shown in human adipose tissue. We report

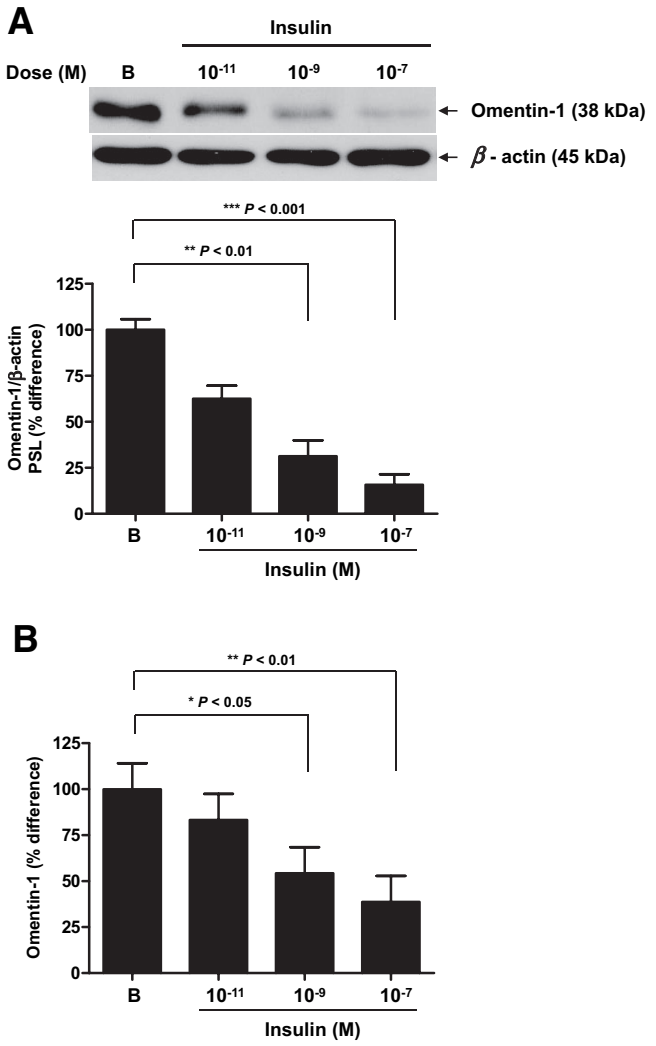

FIG. 3. *A*: Dose-dependent effects of insulin (10^{-11}, 10^{-9}, and 10^{-7} mol/l) in the presence of 5 mmol/l D-glucose on omentin-1 net protein production in control human omental adipose tissue explants at 24 h was assessed by Western blotting. Western blot analysis of protein extracts from omental adipose tissues demonstrate that the antibody against omentin-1 and the antibody against β-actin recognized bands with apparent molecular weights of 38 and 45 kDa, respectively (*A*, *inserts*). Densitometric analysis of omentin-1 immune complexes having normalized to β-actin, respectively, revealed that protein levels of omentin-1 were significantly decreased by insulin (10^{-9} and 10^{-7} mol/l) in control human omental adipose tissue explants. Data are means ± SE of six experiments. Each experiment was carried out with six different samples from six different subjects in three replicates. Group comparison by Friedman's ANOVA and post hoc Dunn's test. *$P < 0.05$; **$P < 0.01$. *B*: Dose-dependent effects of insulin (10^{-11}, 10^{-9}, and 10^{-7} mol/l) in the presence of 5 mmol/l D-glucose on omentin-1 secretion into conditioned media from control human omental adipose tissue explants at 24 h were measured by ELISA. Omentin-1 secretion was significantly decreased by (10^{-9} and 10^{-7} mol/l) from control human omental adipose tissue explants. Data are means ± SE of six experiments. Each experiment was carried out with six different samples from six different subjects in three replicates. Group comparison by Friedman's ANOVA and post hoc Dunn's test. *$P < 0.05$; **$P < 0.01$.

for the first time the expression of omentin-1 in omental human adipose tissues simultaneously at both mRNA and protein levels. Furthermore, we present novel data showing the presence and a significant decrease of adipose tissue omentin-1 mRNA expression and protein levels, respectively, in overweight women with PCOS. In addition, significantly lower plasma omentin-1 levels were detected in these women. More importantly, we describe original observations of the effect of glucose, insulin, and gonadal and adrenal steroids; interestingly, both glucose and insulin caused a significant dose-dependent decrease in omentin-1 net protein production and secretion into conditioned media from control human omental adipose tissue explants. We also show that insulin significantly decreases omentin-1 levels in vivo. Unfortunately, due to technical limitations in omental adipose tissue procurement, we were unable to obtain sufficient amounts of samples/patients to perform stromal vascular separation in omental adipose tissue depots. These limitations not withstanding, it is clear that adipose tissue from our overweight women with PCOS express less omentin-1.

The lower plasma and adipose tissue omentin-1 levels in women with PCOS, an insulin resistance and prodiabetic state, is of interest given that it has recently been reported (10) that obese insulin-resistant subjects had lower plasma omentin-1 levels. In our study, it is unlikely that either BMI or WHR are responsible for lower omentin-1 mRNA expression and protein levels in women with PCOS, given that both groups were matched for these variables. Moreover, like others, we detected a significant negative correlation between plasma omentin-1 and omentin-1 mRNA expression levels in omental human adipose tissue with BMI and WHR (10), although these were no different between PCOS and control subjects. Similar findings were noted with respect to omentin-1 protein levels in omental human adipose tissue depots.

Women with PCOS, particularly those who are overweight, have a higher incidence of insulin resistance (1). We, like others (10), found a significant negative correlation between plasma omentin-1 and HOMA. Of interest, we describe for the first time significant negative correlations between plasma omentin-1 with glucose and also 17β-estradiol levels. Similar observations were noted with respect to omentin-1 mRNA expression and protein levels in omental human adipose tissue depots. However, caution needs to be exercised as these correlations may be spurious, without causative significance, resulting from the simple fact that our women with PCOS had significantly higher fasting serum glucose and 17β-estradiol levels, respectively. In relation to this, Wurm et al. (16) recently reported no significant changes in plasma omentin-1 levels before and 2 h after glucose intake, semiquantified by Western immunoblotting, although, in an ex vivo system, we noted that glucose and insulin caused a significant dose-dependent decrease in omentin-1 net protein production and secretion into condition media, respectively. It is therefore uncertain as to whether the decreased omentin-1 levels observed in our women with PCOS is attributable to insulin resistance per se.

From Study 2, we derive novel observations of a profound decrease by insulin in omentin-1 levels in vivo, thus further supporting our data on the regulation of omentin-1 ex vivo. This effect of insulin appears to be relatively acute, achieving a maximal effect 4 h after commencement of insulin and persisting throughout the entire period of hyperinsulinemia. Taken together, these findings could tentatively explain the decreased levels of omentin-1 seen in our hyperinsulinemic PCOS subjects. Of secondary interest, there appears to be no diurnal variation in omentin-1 levels as depicted in Fig. 5. It should be emphasized that the primary aim of this study was not to investigate the diurnal variation of omentin-1. Also, our study utilized relatively small numbers of subjects because of the challenge imposed by the prolonged insulin-glucose infusion

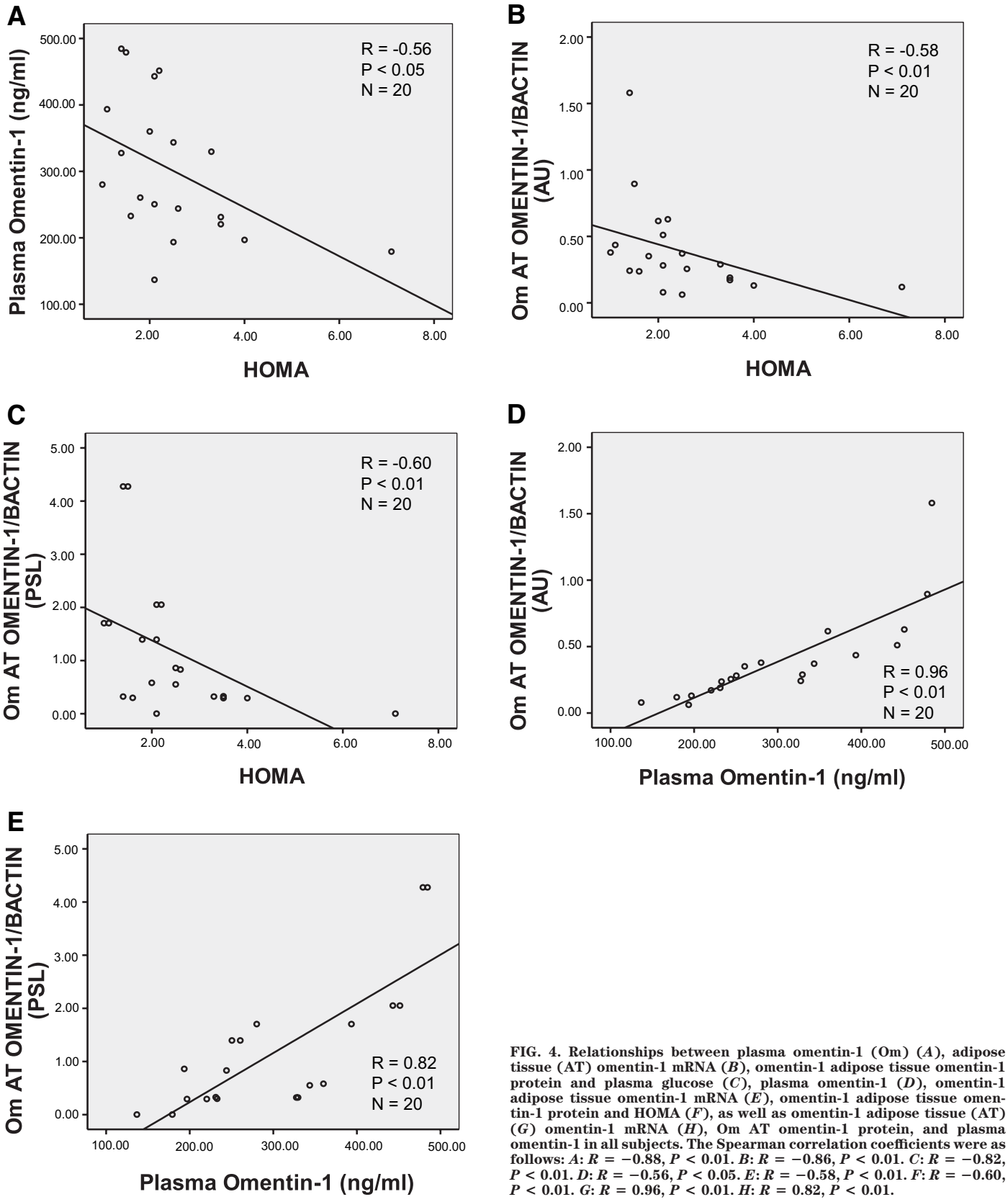

FIG. 4. Relationships between plasma omentin-1 (Om) (*A*), adipose tissue (AT) omentin-1 mRNA (*B*), omentin-1 adipose tissue omentin-1 protein and plasma glucose (*C*), plasma omentin-1 (*D*), omentin-1 adipose tissue omentin-1 mRNA (*E*), omentin-1 adipose tissue omentin-1 protein and HOMA (*F*), as well as omentin-1 adipose tissue (AT) (*G*) omentin-1 mRNA (*H*), Om AT omentin-1 protein, and plasma omentin-1 in all subjects. The Spearman correlation coefficients were as follows: *A*: $R = -0.88$, $P < 0.01$. *B*: $R = -0.86$, $P < 0.01$. *C*: $R = -0.82$, $P < 0.01$. *D*: $R = -0.56$, $P < 0.05$. *E*: $R = -0.58$, $P < 0.01$. *F*: $R = -0.60$, $P < 0.01$. *G*: $R = 0.96$, $P < 0.01$. *H*: $R = 0.82$, $P < 0.01$.

study, and, hence, caution needs to be exercised in interpretation of these results.

Yang et al. (8) showed that treatment with recombinant omentin-1 enhances insulin-stimulated glucose uptake in subcutaneous as well as in human omental adipocytes. They went on further to demonstrate that omentin-1 stimulated Akt phosphorylation in both the absence and presence of insulin. It is well known that insulin-stimulated GLUT4 translocation via activation of Akt signaling is important in maintaining glucose homeostasis (17). In

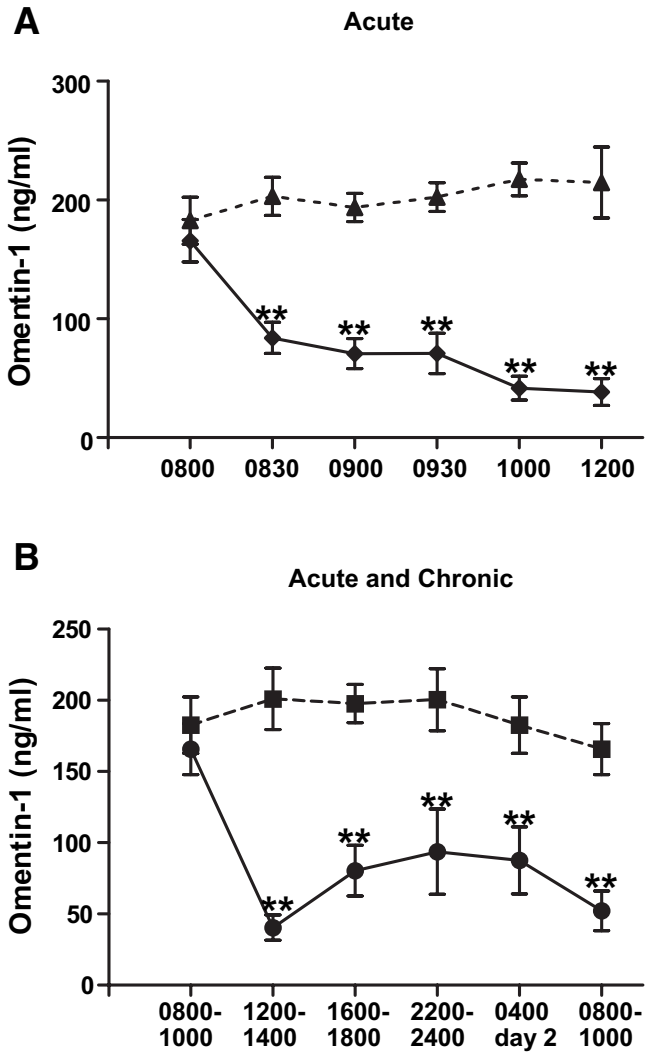

FIG. 5. *A*: Mean concentrations of omentin-1 in ng/ml in all subjects, before and after insulin infusion (acute effects). Data are means ± SD. Group comparison by Mann-Whitney *U* test. **$P < 0.01$. ▲, Control; ♦, Infusion. *B*: Mean concentrations of omentin-1 in ng/ml in all subjects, before and after insulin infusion (acute and chronic effects). Data are means ± SD. Group comparison by Mann-Whitney *U* test. **$P < 0.01$. ■, control; ●, infusion.

relation to this, we have recently shown that GLUT4 content in adipose tissue is significantly lower in women with PCOS (18). Additionally, Rosenbaum et al. (19) showed that women with PCOS have decreased sensitivity and responsiveness to insulin associated with diminished GLUT4 content in adipocytes. Thus, given omentin-1's insulin-sensitizing effect as outlined above, we tentatively hypothesize that the decreased omentin-1 levels in adipose tissue of women with PCOS as in our study may explain, in part, the corresponding decreased levels of GLUT4 observed in adipose tissue and adipocytes of women with PCOS, possibly as a consequence of the hyperinsulinemic state seen in these women. However, our speculations need to be further investigated. It is important to bear in mind that the regulation of omentin-1 in adipose tissue is probably multifactorial. Moreover, it would be of interest to know whether or not the effects of insulin on omentin-1 production are also applicable to other tissues given our in vivo data. Further studies are needed to elucidate the role of other factors that regulate omentin-1 production.

A limitation of our study may relate to the number of subjects studied. However, obtaining BMI/WHR matched and menstrual cycle–synchronized blood and tissue samples impeded subject recruitment. Not withstanding, our observations are highly consistent and significant and raise interesting questions on the mechanisms regulating omentin-1 expression. Moreover, a sample size as in our study is only likely to detect differences that are enormous/significant. Finally, it should be emphasized that our findings relate only to overweight women with PCOS, and it would be of interest to study omentin-1 levels in lean women with PCOS.

In conclusion, we present novel data of decreased plasma omentin-1 levels as well as decreased expression of omentin-1 mRNA and protein levels in omental adipose tissue of women with PCOS. More importantly, we show for the first time the potent and robust regulation of omentin-1 ex vivo and in vivo by glucose and insulin. The physiologic and pathologic significance of our findings remain to be elucidated but may indicate a mechanism for the development of insulin resistance in women with PCOS.

ACKNOWLEDGMENTS

The General Charities of the City of Coventry funded this study.

H.S.R. acknowledges S. Waheguru, University of Warwick, for his continual support.

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