Omentin-1, a Novel Adipokine, is decreased in Overweight Insulin Resistant Women with the Polycystic Ovary Syndrome: ex vivo and in vivo Regulation of Omentin-1 by Insulin and Glucose

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Running Title: Insulin & Glucose Decrease Omentin-1

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ABSTRACT

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 exists on the relative expression and regulation of omentin-1 in adipose tissue (AT) of PCOS women.

Objectives: To assess mRNA and protein levels of omentin-1 in omental (om) AT of PCOS women and matched controls, including circulating omentin-1. Ex vivo and in vivo regulation of AT 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 ELISA. The effects of D-glucose, insulin, gonadal and adrenal steroids on AT omentin-1 were analysed 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 PCOS women ($P < 0.05$), compared to controls, there was significantly lower levels of omentin-1 mRNA ($P < 0.01$) and protein ($P < 0.05$) in om AT of PCOS women ($P < 0.01$). Furthermore, in om AT 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 down regulated by insulin and glucose. These may in part explain the decreased omentin-1 levels observed in our overweight PCOS women.

KEYWORDS. Omentin-1, PCOS, adipose tissue, adipocyte, adipokine, insulin resistance, metabolic syndrome, insulin, glucose
Polycystic ovary syndrome (PCOS), a common endocrinopathy affecting 5-10% of women in the reproductive age, is characterised by irregular menses, hyperandrogenism and associated with insulin resistance (IR) and pancreatic β-cell dysfunction, impaired glucose tolerance (IGT), type 2 diabetes mellitus (T2DM), dyslipidemia and visceral obesity (1, 2). The consequent hyperinsulinemia, is more prevalent in lean and obese women with PCOS whence compared to 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 cardio-metabolic risk than sc adipose tissue (5) as removal of visceral rather than sc 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 on the increased levels of visfatin in PCOS women; 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 (7).

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

As PCOS is a pro-diabetic 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 sc and om adipose tissue depots in these women against age, BMI and waist-hip ratio matched controls. With PCOS being a state of hyperinsulinemia with altered gonadal and adrenal steroid levels, we also assessed the effects of glucose with and without insulin as well as these steroid hormones on omentin-1 secretion into conditioned media as well as mRNA expression and protein levels from human om adipose tissue explants. Finally, we studied the effects of hyperinsulinemia on plasma omentin-1 levels via a prolonged insulin-glucose infusion in healthy subjects.

**SUBJECTS AND METHODS**

**Subjects.**

**Study 1.** All PCOS patients met all 3 criteria of the revised 2003 Rotterdam ESHRE/ASRM PCOS Consensus Workshop Group diagnostic criteria. The 3 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 US scan, 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 over 40 yr, known
cardiovascular disease, thyroid disease, neoplasms, current smoking, diabetes mellitus, hypertension (blood pressure, >140/90 mmHg), renal impairment (serum creatinine, >120 µmol/L). None of these women were on any medications for at least 6 months prior to the study, including oral contraceptives, glucocorticoids, ovulation induction agents, anti-diabetic and anti-obesity drugs, estrogenic, anti-androgenic or anti-hypertensive medication. Also, the presence of other endocrinopathies were ruled out by measuring basal serum 17-hydroxyprogesterone, prolactin, and by measuring 0800-0900 hr cortisol after 1.0 mg (2300 hr) overnight dexamethasone suppression (value below 30nmol/l was considered to rule out Cushing’s syndrome).

All subjects suppressed cortisol below 30nmol/l.

After an overnight fast, blood samples, sc and om adipose tissue were obtained (0800-1000 hrs) from adult female patients undergoing elective surgery for infertility investigation. Subjects were initially seen at the infertility clinic and then scheduled for laparoscopy in order to assess Fallopian tube(s) patency. All subjects underwent anthropometric measurements i.e. weight, height and waist to hip circumference ratio (WHR). A total of 51 subjects were recruited consecutively from the infertility clinic in accordance with the inclusion/exclusion criteria (PCOS: n = 13; Controls: n = 38). Of the 13 PCOS subjects recruited, 3 withdrew before the study could be completed. In the control group, 4 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). Sc 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 (day 2-4 from the first day of 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, UK) for primary adipose tissue culture. All patients underwent anthropometric measurements i.e. weight, height and waist to hip circumference ratio (WHR). The Local Research Ethics Committee approved the study and all patients involved gave their informed consent, in accordance with the guidelines in The Declaration of Helsinki 2000.

Study 2. We measured omentin-1 in six healthy subjects [age: (mean ± SD): 26.5 ± 8 years, BMI: 23.2 ± 2.5 kg/m²]. None of these subjects were on any medications for at least 6 months prior to the study. In order 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 minute intervals from 0800 to 1000 hours. Subsequently, non-fasting omentin-1 levels were measured at 2-hourly intervals until 2400 hours and then at 0400 hours as well as at 30 minute intervals from 0800 to 1000 hours on day 2. On the following day the same subjects were subjected to a prolonged insulin-glucose infusion for 26 hours beginning at 0800 hours. Insulin (Human Actrapid) was administered intravenously as a priming dose of 0.04 U/kg followed by continuous infusion of 0.5 mU/kg/minutes. By choosing this rate of insulin infusion we expected to achieve hyperinsulinemia with an approximate four to six fold 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 minute intervals between 0800 and 1000 hours on day 1 and day 2 of the prolonged insulin-glucose infusion (the first and the last two hours of the infusion).
Intermediate blood samples (non-fasted) were taken at 2-hourly intervals until 2400 h and then at 0400 h on day 2. Pre-prandial glucose levels were maintained between 4·0 and 6·0 mmol/l. The Local Research Ethics Committee approved the study and all patients involved gave their informed consent, in accordance with the guidelines in The Declaration of Helsinki 2000.

**Biochemical and hormonal analysis.** Assays for glucose, insulin, luteinizing hormone (LH), follicular stimulating hormone (FSH), 17β-estradiol (E₂), progesterone, testosterone, androstenedione, dehydroxyepiandrosterone-sulphate (DHEA-S) and sex hormone binding globulin (SHBG) 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 \[ \text{HOMA} = \frac{I_o \times G_o}{22.5} \] where \( I_o \) is the fasting insulin and \( G_o \) is the fasting glucose, as described by Matthews *et al.* (13).

Omentin levels in plasma and conditioned media from human om adipose tissue explants were measured using a commercially available ELISA (ELISA kit: AXXORA, Nottingham, UK), according to manufacturer’s protocol, with an intra-assay coefficient of variation of less than 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 to 3 g of adipose tissue was minced into 5-10 mg (~1 mm³) fragments, washed with a 230 µm mesh (Filter no. 60, Sigma-Aldrich, Gillingham, UK)) and rinsed with sterile PBS warmed to 37°C. Samples were then transferred to six well plates (~50mg/well) containing 3 ml of Media 199 (Gibco-BRL) supplemented with 50 µg/ml gentamicin and 1 % FBS (containing insulin at a concentration of 10⁻¹⁴ M) and cultured for 24 hours 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% CO₂/95% 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, UK). The purity of the extracted RNA was measured by a NanoDrop spectrophotometer. A set concentration of RNA was reverse transcribed into cDNA, by using M-MuLV Reverse Transcriptase (Fermentas, York, UK) and random hexamers (Promega, Southampton, UK) as primers.

**Reverse Transcriptase Polymerase Chain Reaction (RT-PCR).** Quantitative PCR of Omentin-1 was performed on a Roche Light Cycler™ system (Roche Molecular Biochemicals, Mannheim, Germany). PCR reactions were carried out in a reaction mixture consisting of 5.0 µl reaction buffer and 2.0mM MgCl₂ (Biogene, Kimbolton, U.K.), 1.0 µl of each primer (10ng/µl), 2.5 µl of cDNA and 0.5 µl of Light Cycler DNA Master SYBR® Green I (Roche, Manheim, Germany). Protocol conditions consisted of denaturation of 95°C for 15 seconds, followed by 40 cycles of 94°C for 1 sec, 58°C for 10 seconds and 72°C for 12 seconds, followed by melting curve analysis. For analysis, quantitative amounts of genes of interest were standardised against the housekeeping gene β-actin. The RNA levels were expressed as a ratio, using “Delta-delta method” for comparing relative expression results between treatments in real-time PCR (15). The sequences of the sense and anti-sense primers used were: Omentin-1 (199bp) 5’- AACAGCTCCCTGCTGAGGTA-3’ and 5’-GCTGGCCATAGGGTGAGTAA-3’; β-actin (216bp) 5’-AAGAGAGGCATCCTCACCCCT-3’ and 5’-TACATGGCTGGGGTCTTGAA-3’.
10μl of the reaction mixture(s) were subsequently electrophoresed on a 1% agarose gel and visualised by ethidium bromide, using a 1 kb DNA ladder (Gibco-BRL, Paisley, UK) 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 the cDNA. RNAs was assayed from three independent biological replicates.

**Western Blotting.** Protein lysates were prepared by homogenising adipose tissue in radioimmunoprecipitation (RIPA) lysis buffer (Upstate, Lake Placid, NY, USA) according to manufacturer’s instructions. Protein samples (30μg/lane) containing SDS-sample buffer (5 M urea, 0.17 M SDS, 0.4 M dithiothreitol, and 50mM Tris-HCl, pH 8.0) were subjected to SDS-polyacrylamide gel electrophoresis (10% resolving gel) and transferred to polyvinylidene difluoride (PVDF) membranes. The PVDF membranes were incubated with primary mouse-anti-human antibody for Omentin-1 (AXXORA, Nottingham, UK) [1:1000 dilution] or primary rabbit-anti-human antibody for beta-actin (Cell Signalling Technology Inc., Beverly, MA, USA) [1:1000 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, UK) [1:2000] for one hour at room temperature. Antibody complexes were visualized using chemiluminescence (ECL+; Amersham, Little Chalfont, Buckinghamshire, UK). Human Omentin-1 peptide (AXXORA, Nottingham, UK) was used as the positive control and water as the negative control (data not shown).

**Statistics.** Non-parametric tests were used. Data are presented as means ± SEM 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 Corporation, Frederick, MD, USA). 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 PCOS and control women. Glucose, insulin, HOMA, triglycerides, 17β-estradiol, testosterone, androstenedione levels and free androgen index (FAI) were significantly higher, whereas sex hormone binding globulin (SHBG) was significantly lower, in PCOS women. ELISA analysis of plasma omentin-1 levels revealed that PCOS patients had significantly lower levels when compared to controls (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 PCOS women. 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 sub adipose tissue samples (data not shown). Real-time RT-PCR analysis corrected over-actin showed a significant decrease of omentin-1 in om (**) *P < 0.01) adipose tissues of PCOS women when compared to normal controls (Figure 1A). The changes noted at mRNA level were also reflected at protein level in PCOS women i.e. significantly lower omentin-1 levels in om adipose tissues of PCOS women (Figure 1B; *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 om adipose tissue explants. Omentin-1 net protein production was significantly decreased dose dependently by D-glucose (20 mmol/L, 40 mmol/L) from control human om adipose tissue explants (Figure 2A: \*P < 0.05, \**P < 0.01, respectively). Similar observations were noted with respect to secretion of omentin-1 into conditioned media in corresponding AT explants (Figure 2B: \*P < 0.05, \**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 om adipose tissue explants (Figure 3A & 3B: \*P < 0.05, \**P < 0.01, respectively). Of note, a similar pattern of omentin-1 regulation by insulin was observed at higher concentrations of D-glucose (30mmol/L) [data not shown].

Effects of testosterone, 17β-estradiol, androstenedione and DHEA-S on omentin-1 levels in control human om adipose tissue explants. Given that PCOS women 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, om adipose tissue omentin-1 mRNA expression as well as 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 analysed individually (Table 2; see online appendix available at http://diabetes.diabetesjournals.org).

Given the observation that glucose and insulin decreased omentin-1 net protein production and secretion into conditioned media (Figure 2A, 2B, 3A & 3B; Spearman Rank analyses demonstrated that plasma omentin-1 significantly negatively correlated with HOMA (Figure 4A: R = -0.56, P < 0.05), but also om adipose tissue mRNA expression (Figure 4B: R = -0.58, P < 0.01) and om adipose tissue protein levels (Figure 4C: R = -0.60, P < 0.01). Furthermore, om adipose tissue mRNA expression and protein levels significantly positively correlated with plasma omentin-1 (Figure 4D & 4E: R = 0.96, P < 0.01; R = 0.82, P < 0.01, respectively). Once again, these findings were consistent when the groups were analysed 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 ± 12pmol/L to 294.6 ± 31pmol/L. Insulin levels remained elevated until the end of the prolonged insulin-glucose infusion (366.0 ± 37pmol/L), thus achieving our objective of an approximate four to six fold 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 ± 17ng/ml at 0800 hours to 205 ± 126ng/ml at 1000 hours the next day (Figure 5A & 5B; P > 0.05).

There was, however, a potent repressive effect of insulin on omentin-1 levels over 26 hours of insulin infusion: from 165 ± 44ng/ml at 0800 hours to 37 ± 14ng/ml at 1000 hours the following day (Figure 5A & 5B; P > 0.05). Of interest, the initial decline in circulating omentin-1 level was relatively acute achieving a nadir at 4 hours.
DISCUSSION

Omentin-1 mRNA expression, a new adipokine (10), has recently been shown in human adipose tissue. We report for the first time the expression of omentin-1 in om 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 PCOS women. 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, 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 om adipose tissue explants. We also show that insulin significantly decreases omentin-1 levels in vivo. Unfortunately, due to technical limitations in om adipose tissue procurement, we were unable to obtain sufficient amounts of sample/patient to perform stromal vascular separation in om adipose tissue depots. These limitations notwithstanding, it is clear that adipose tissue from our overweight PCOS women express less omentin-1.

The lower plasma and adipose tissue omentin-1 levels in women with PCOS, an IR and pro-diabetic state is of interest given that it has recently been reported that obese insulin-resistant subjects had lower plasma omentin-1 levels (10). In our study, it is unlikely that either BMI or WHR are responsible for lower omentin-1 mRNA expression and protein levels in PCOS women, 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 om human adipose tissue with BMI and WHR (10), although these were no different between PCOS and controls. Similar findings were noted with respect to omentin-1 protein levels in om human adipose tissue depots.

Women with PCOS, particularly those who are overweight, have a higher incidence of IR (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 correlation 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 om 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 PCOS women had significantly higher fasting serum glucose and 17β-estradiol levels, respectively. In relation to this, Wurm et al. recently reported no significant changes in plasma omentin-1 levels before and 2 hours after glucose intake, semi-quantified by western immunoblotting (16), 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 or not the decreased omentin-1 levels observed in our PCOS women 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 hours 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 Figure 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 study and hence caution needs to be
exercised in interpretation of these results.

Yang et al. showed that treatment with
recombinant omentin-1 enhances insulin
stimulated glucose uptake in sc as well as in
human omental adipocytes. They went on
further to demonstrate that omentin-1
stimulated Akt phosphorylation both in the
absence and presence of insulin (8). It is well
known that insulin stimulated GLUT4
translocation via activation of Akt signalling
is important in maintaining glucose
homeostasis (17). In relation to this, we have
recently shown that GLUT4 content in
adipose tissue is significantly lower in PCOS
women (18). Additionally, Rosenbaum et al.
showed that PCOS women have decreased
sensitivity and responsiveness to insulin
associated with diminished GLUT4 content in
adipocytes (19). Thus, given omentin-1’s
insulin-sensitizing effect as outlined above,
we tentatively hypothesize that the decreased
omentin-1 levels in adipose tissue of PCOS
women as in our study may explain, in part,
the corresponding decreased levels of GLUT4
observed in adipose tissue and adipocytes of
PCOS women; 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 synchronised 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 emphasised that our
findings relate only to overweight PCOS
women, and it would be of interest to study
omentin-1 levels in lean PCOS women.

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 om adipose tissue of
PCOS women. 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.

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**TABLE 1.** Clinical, hormonal and metabolic features of women with PCOS and controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>PCOS (n = 10)</th>
<th>Controls (n = 10)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>32.2 ± 5.8</td>
<td>32.7 ± 4.5</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.9 ± 2.3</td>
<td>29.2 ± 2.6</td>
<td>NS</td>
</tr>
<tr>
<td>WHR</td>
<td>0.87 ± 0.15</td>
<td>0.85 ± 0.16</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.2 ± 0.7</td>
<td>4.6 ± 0.5</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>90.9 ± 38.8</td>
<td>52.4 ± 15.0</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>HOMA</td>
<td>3.5 ± 1.8</td>
<td>1.8 ± 0.6</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>5.0 ± 0.8</td>
<td>5.2 ± 0.8</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting triglycerides (mmol/L)</td>
<td>2.2 ± 1.2</td>
<td>1.0 ± 0.4</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>LH (IU/L)</td>
<td>8.6 ± 4.3</td>
<td>6.0 ± 4.2</td>
<td>NS</td>
</tr>
<tr>
<td>FSH (IU/L)</td>
<td>6.3 ± 1.6</td>
<td>6.4 ± 2.7</td>
<td>NS</td>
</tr>
<tr>
<td>Prolactin (mU/L)</td>
<td>357.9 ± 87.9</td>
<td>298.0 ± 85.8</td>
<td>NS</td>
</tr>
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<td>E₂ (pmol/L)</td>
<td>365.4 ± 101.0</td>
<td>207.5 ± 67.8</td>
<td>P &lt; 0.01</td>
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<tr>
<td>Progesterone (nmol/L)</td>
<td>1.9 ± 0.6</td>
<td>2.1 ± 0.4</td>
<td>NS</td>
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<tr>
<td>17-OH-P (nmol/L)</td>
<td>2.5 ± 0.7</td>
<td>1.9 ± 0.6</td>
<td>NS</td>
</tr>
<tr>
<td>Testosterone (nmol/L)</td>
<td>3.9 ± 1.1</td>
<td>2.4 ± 0.5</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Androstenedione (nmol/L)</td>
<td>13.0 ± 3.4</td>
<td>8.6 ± 2.3</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>DHEA-S (μmol/L)</td>
<td>6.0 ± 1.3</td>
<td>4.7 ± 1.1</td>
<td>NS</td>
</tr>
<tr>
<td>SHBG (nmol/L)</td>
<td>31.6 ± 6.9</td>
<td>53.1 ± 16.6</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>FAI</td>
<td>12.3 ± 5.3</td>
<td>4.5 ± 8.1</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Omentin-1 (ng/ml)</td>
<td>255.8 ± 78.2</td>
<td>348.0 ± 112.6</td>
<td>P &lt; 0.05</td>
</tr>
</tbody>
</table>

Free Androgen Index (FAI) = T (nmol/liter)/SHBG (nmol/liter) x 100

Data are means ± SD. Group comparison by Mann-Whitney U test.

NS = not significant

N = 10 each group
FIGURE LEGENDS

Figure 1. (A) Omentin-1 mRNA expression relative to β-actin is significantly decreased in human omental (om) adipose tissue depots when comparing all PCOS women to all normal controls, using real-time RT-PCR. Data are means ± SEM. 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 om adipose tissue depots when comparing all PCOS women to all normal controls. Data are means ± SEM. Group comparison by Mann-Whitney U test. *P < 0.05. PSL, phospho-stimulated light units.

Figure 2. (A) Dose dependent effects of D-glucose (5mmol/L, 10mmol/L, 20mmol/L, 40mmol/L) on omentin-1 net protein production in control human om adipose tissue explants at 24 hours was assessed by western blotting. Western blot analysis of protein extracts from omental (om) adipose tissues demonstrate that the antibody against omentin-1 and the antibody against β-actin recognised bands with apparent molecular weights of 38kDa and 45kDa, respectively (Figure 2A-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 (20mmol/L, 40mmol/L) in control human om adipose tissue explants. Data are means ± SEM 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 om adipose tissue explants at 24 hours were measured by ELISA. Omentin-1 secretion was significantly decreased by D-glucose (20mmol/L, 40mmol/L) from human om adipose tissue explants. Data are means ± SEM 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.

Figure 3. (A) Dose dependent effects of insulin (10⁻¹¹ M, 10⁻⁹ M, 10⁻⁷ M) in the presence of 5mmol/L D-glucose on omentin-1 net protein production in control human om adipose tissue explants at 24 hours was assessed by western blotting. Western blot analysis of protein extracts from omental (om) adipose tissues demonstrate that the antibody against omentin-1 and the antibody against β-actin recognised bands with apparent molecular weights of 38kDa and 45kDa, respectively (Figure 3A-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 M, 10⁻⁷ M) in control human om adipose tissue explants. Data are means ± SEM 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 M, 10⁻⁹ M, 10⁻⁷ M) in the presence of 5mmol/L D-glucose on omentin-1 secretion into conditioned media from control human om adipose tissue explants at 24 hours were measured by ELISA. Omentin-1 secretion was significantly decreased by (10⁻⁹ M, 10⁻⁷ M) from control human om adipose tissue explants. Data are means ± SEM 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.
**Figure 4.** Relationships between (A) plasma omentin-1 (B) Om adipose tissue (AT) omentin-1 mRNA, (C) Om AT omentin-1 protein and plasma glucose, (D) plasma omentin-1 (E) Om adipose tissue (AT) omentin-1 mRNA, (F) Om AT omentin-1 protein and HOMA as well as (G) Om adipose tissue (AT) 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].

**Figure 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. (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.
FIGURE 1

A

Omental

Omentin-1/β-actin mRNA expression (% difference)

Normal  PCOS

B

Omental

Omentin-1/β-actin

Normal  PCOS

P.S. (% difference)
Insulin & Glucose Decrease Omentin-1

FIGURE 2

A

<table>
<thead>
<tr>
<th>Glucose (mmol/L)</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>40</th>
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</thead>
<tbody>
<tr>
<td>Omentin-1 (35 kDa)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>β-actin (48 kDa)</td>
<td></td>
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</tbody>
</table>

*B P < 0.05

** P < 0.01

B

<table>
<thead>
<tr>
<th>Glucose (mmol/L)</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omentin-1 (%)</td>
<td></td>
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</tr>
</tbody>
</table>

*B P < 0.05

** P < 0.01
FIGURE 3

A

B

Insulin & Glucose Decrease Omentin-1
FIGURE 4

A

Plasma Omentin-1 (ng/ml)

HOMA

R = 0.58
P < 0.05
N = 20

B

Om AT OMENTIN-1/BACTIN (AU)

HOMA

N = 20

C

Om AT OMENTIN-1/BACTIN (PSL)

HOMA

R = 0.83
P < 0.01
N = 20

D

Om AT OMENTIN-1/BACTIN (AU)

N = 20

E

Om AT OMENTIN-1/BACTIN (PSL)

Plasma Omentin-1 (ng/ml)

R = 0.82
P < 0.01
N = 20