

# Adiponectin is independently associated with insulin sensitivity in women with polycystic ovary syndrome

Joachim Spranger\*, Matthias Möhlig\*, Uta Wegewitz\*, Michael Ristow\*, Andreas F. H. Pfeiffer\*, Thilo Schill†, Hans W. Schlösser‡, Georg Brabant‡ and Christof Schöfl‡

\*Department of Clinical Nutrition, German Institute of Human Nutrition, Nuthetal and Department of Endocrinology, Diabetes and Nutrition, Charité-University Medicine Berlin, Campus Benjamin Franklin, Berlin and Departments of †Reproduction and Fertility and ‡Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, Hannover, Germany

(Received 25 March 2004; returned for revision 12 May 2004; finally revised 9 July 2004; accepted 5 October 2004)

## Summary

**OBJECTIVE** The polycystic ovary syndrome (PCOS) is associated with obesity and insulin resistance predisposing to diabetes mellitus type 2 and atherosclerosis. Adiponectin is a recently discovered adipocytokine with insulin-sensitizing and putative antiatherosclerotic properties. The aim of the study was to elucidate determinants of circulating adiponectin levels and to investigate the potential role of adiponectin in insulin resistance in PCOS women.

**PATIENTS AND MEASUREMENTS** Plasma adiponectin and parameters of obesity, insulin resistance and hyperandrogenism were measured in 62 women with PCOS and in 35 healthy female controls.

**RESULTS** Both in PCOS and controls, adiponectin levels were lower in overweight or obese women than in normal-weight women, without any difference between PCOS and controls after adjustment for body mass index (BMI). In PCOS and in controls there was a significant correlation of adiponectin with BMI ( $r = -0.516$ ,  $P < 0.001$ ), fasting insulin ( $r = -0.404$ ,  $P < 0.001$ ), homeostasis model sensitivity (HOMA %S) ( $r = -0.424$ ,  $P < 0.001$ ) and testosterone ( $r = -0.279$ ,  $P < 0.01$ ), but no correlation with androstenedione ( $r = -0.112$ ,  $P = 0.325$ ),

17-OH-progesterone ( $r = -0.031$ ,  $P = 0.784$ ) or the LH/FSH ratio ( $r = -0.033$ ,  $P = 0.753$ ). Multiple linear regression analysis revealed that BMI and HOMA %S but not testosterone were independently associated with adiponectin plasma levels, explaining 16% (BMI) and 13% (HOMA %S) of the variability of adiponectin, respectively. In PCOS patients insulin sensitivity, as indicated by continuous infusion of glucose with model assessment (CIGMA %S) was significantly correlated with adiponectin ( $r = 0.55$ ;  $P < 0.001$ ), BMI ( $r = -0.575$ ;  $P < 0.001$ ), waist-to-hip ratio (WHR) ( $r = -0.48$ ;  $P = 0.001$ ), body fat mass assessed by dual-energy X-ray-absorptiometry (DEXA) [Dexa-fat (total) ( $r = -0.61$ ;  $P < 0.001$ ) and Dexa-fat (trunk) ( $r = -0.59$ ;  $P < 0.001$ )] and with testosterone ( $r = -0.42$ ;  $P = 0.001$ ). Multiple linear regression analysis demonstrated that markers of obesity such as BMI, total or truncal fat mass, age and adiponectin were independently associated with CIGMA %S, and that circulating adiponectin accounted for about 18% of the degree of insulin resistance in PCOS. By contrast, testosterone was not a significant factor, suggesting that PCOS *per se* did not affect insulin sensitivity independent from obesity, age and adiponectin. Metformin treatment for 6 months in insulin-resistant PCOS women ( $n = 9$ ) had no effect on plasma adiponectin ( $P = 0.59$ ) despite significant loss of weight and fat mass and improvement in hyperandrogenaemia. **CONCLUSIONS** PCOS *per se* is not associated with decreased levels of plasma adiponectin. However, circulating adiponectin is independently associated with the degree of insulin resistance in PCOS women and may contribute to the development and/or maintenance of insulin resistance independent from adiposity.

The polycystic ovary syndrome (PCOS) is one of the most frequent endocrine disorders in women. It is clinically characterized by hirsutism, chronic anovulation and infertility (Franks, 1995; Dunaif, 1997; Lobo & Carmina, 2000). In addition to the reproductive abnormalities a significant proportion of PCOS women are predisposed to adiposity and insulin resistance (Franks, 1995; Dunaif, 1997; Lobo & Carmina, 2000). Amelioration of metabolic abnormalities, especially of insulin resistance, by lifestyle or pharmacological intervention can improve hyperandrogenism and fertility (Pasquali & Gamberini, 2002; Costello & Eden,

Correspondence: Christof Schöfl, Abteilung Gastroenterologie, Hepatologie und Endokrinologie, Medizinische Hochschule Hannover, Carl-Neubergstr. 1, D-30623 Hannover, Germany. Tel.: (49) 511 5326528; Fax: (49) 511 5323825; E-mail: schoefl.christof@mh-hannover.de

2003; De Leo *et al.*, 2003; Haas *et al.*, 2003; Harborne *et al.*, 2003), indicating a close link between the reproductive and metabolic disturbances in PCOS. Obesity and insulin resistance, however, are also well-known risk factors for developing type 2 diabetes mellitus and cardiovascular disease (Warram *et al.*, 1997). Several studies have consistently demonstrated a high risk for impaired glucose tolerance and type 2 diabetes mellitus, as well as atherosclerosis and cardiovascular disease in PCOS women (Conway *et al.*, 1992; Guzick *et al.*, 1996; Birdsall *et al.*, 1997; Ehrmann *et al.*, 1999; Legro *et al.*, 1999; Lobo & Carmina, 2000). However, the mechanisms linking PCOS to these metabolic and vascular changes are not completely understood.

There is growing evidence that differential expression of adipocyte-derived cytokines such as tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-6 (IL-6), and others may provide a link between obesity, insulin resistance, type 2 diabetes and cardiovascular disease (Hotamisligil & Spiegelman, 1994; Alessi *et al.*, 1997; Festa *et al.*, 2000; Yudkin *et al.*, 2000; Pradhan *et al.*, 2001; Freeman *et al.*, 2002; Spranger *et al.*, 2003b). A novel adipocytokine called adiponectin has been described (Scherer *et al.*, 1995; Hu *et al.*, 1996; Maeda *et al.*, 1996; Nakano *et al.*, 1996). Adiponectin is exclusively and abundantly expressed in white adipose tissue, and shares high structural homology with collagen VIII, X and complement C1q (Shapiro & Scherer, 1998). It plays a physiological role in the control of insulin sensitivity (Ouchi *et al.*, 2000; Berg *et al.*, 2001; Yamauchi *et al.*, 2001) and some recent studies have demonstrated that high levels of adiponectin may protect against the development of type 2 diabetes (Lindsay *et al.*, 2002; Spranger *et al.*, 2003a). A number of studies point to an additional antiatherogenic action (Ouchi *et al.*, 1999; Kubota *et al.*, 2002). This suggests that adiponectin could play an important and integrative role in the pathogenesis of obesity, insulin resistance, type 2 diabetes and cardiovascular disease (Hotta *et al.*, 2000; Berg *et al.*, 2001, 2002; Combs *et al.*, 2001; Yamauchi *et al.*, 2001; Kubota *et al.*, 2002; Maeda *et al.*, 2002; Spranger *et al.*, 2003a).

Recent studies reported that hypoadiponectinaemia in women with PCOS is predominantly correlated with the degree of obesity (Ducluzeau *et al.*, 2003; Orio *et al.*, 2003; Panidis *et al.*, 2003). In normal subjects and in non-PCOS patients, however, there is a close link between adiponectin plasma levels and various indicators of insulin resistance (Weyer *et al.*, 2001; Yamamoto *et al.*, 2002; Pellme *et al.*, 2003; Tschritter *et al.*, 2003). As in PCOS patients the degree of insulin resistance is not necessarily and entirely explained by the degree of obesity; the precise interaction between adiponectin, insulin resistance and obesity appears yet to be defined in PCOS women. In addition, PCOS-associated hyperandrogenaemia may further modulate plasma adiponectin (Nishizawa *et al.*, 2002), which could provide a potential mechanism whereby PCOS-related hyperandrogenaemia enhances the susceptibility of PCOS

women to the insulin resistance syndrome and its long-term complications.

To further elucidate the interactions between adiponectin, obesity, insulin resistance and hyperandrogenaemia, and to investigate a potential role of adiponectin in insulin resistance in PCOS, we measured plasma adiponectin and parameters of obesity, insulin resistance and hyperandrogenism in 62 women with PCOS and compared them to 35 healthy female controls. Finally, nine insulin-resistant PCOS women were treated with metformin for 6 months and the effects on body weight, insulin resistance and hyperandrogenaemia were correlated with changes in circulating plasma adiponectin.

## Subjects and methods

### Subjects

Sixty-two consecutive women with PCOS were included in the study after informed consent was obtained. The diagnosis of PCOS was based on (1) the presence of chronic ovulatory dysfunction, that is oligomenorrhoea (four or less cycles in the last 6 months) or amenorrhoea (no cycles in the last 6 months), and (2) clinical signs of hyperandrogenism (i.e. hirsutism or acne) or (3) laboratory findings, that is hyperandrogenaemia, defined as serum androgen levels [dehydroepiandrosterone sulfate (DHEAS), 17-OH-progesterone, androstenedione or total testosterone] above the upper limit of normal for the respective assay and/or elevated LH/FSH ratio, and (4) the exclusion of other disorders such as Cushing's syndrome, late-onset 21-hydroxylase deficiency, thyroid dysfunction, hyperprolactinaemia or androgen-secreting tumours. These diagnostic criteria for PCOS are consistent with the most commonly used diagnostic criteria for PCOS, often referred to as the NIH consensus criteria (Zawadzki & Dunaif, 1992). The clinical and endocrine features of the women are given in Table 1. Two patients were diagnosed with Hashimoto's thyroiditis. They were euthyroid under thyroid hormone replacement therapy. All other patients were free of other diseases and were taking no medication. Nine obese and insulin-resistant PCOS women were re-evaluated after 6 months of treatment with 850 mg metformin thrice daily.

All women were studied within the first 10 days following menstruation in case of mild oligomenorrhoea, or at random times if they suffered severe oligo- or amenorrhoea. Blood was sampled in the morning after an overnight fast and the samples were stored at  $-20^{\circ}\text{C}$  until analysis. Insulin resistance was assessed by the homeostasis model (HOMA), using the mean of three fasting glucose and insulin levels, and by a 2-h continuous infusion of glucose with model assessment (CIGMA) in all patients (Hermans *et al.*, 1999). The values were calculated using the computer program HOMA-CIGMA version 2 kindly provided by Dr J. C. Levy (Levy *et al.*, 1998). Women were regarded as insulin resistant if the calculated sensitivity (%S) according to

**Table 1** Clinical and endocrine features of PCOS patients and healthy controls

Variable	Controls* (n = 35)	PCOS* (n = 62)	P-value†
Age (years)	30.4 ± 1.0	28.9 ± 0.6	0.240
BMI (kg/m <sup>2</sup> )	25. ± 1.0	30.6 ± 0.9	0.001
WHR	0.78 ± 0.01	0.80 ± 0.01	0.058
Fasting glucose (mmol/l)	5.0 ± 0.1	4.4 ± 0.1	0.001
Fasting insulin (pmol/l)	56.3 ± 5.2	88.7 ± 7.1	< 0.001
HOMA (%S)	111 ± 6	86 ± 7	0.001
LH/FSH	0.99 ± 0.08	2.00 ± 0.30	0.001
E <sub>2</sub> (pmol/l)	177.6 ± 24.2	251.0 ± 43.7	0.687
Progesterone (nmol/l)	2.37 ± 0.29	4.45 ± 1.28	0.107
Testosterone (nmol/l)	1.70 ± 0.14	3.33 ± 0.17	< 0.001
SHBG (nmol/l)	77.5 ± 8.6	60.8 ± 6.3	0.068
Androstenedione (nmol/l)	5.17 ± 3.11	8.27 ± 0.38	< 0.001
17-OH-Progesterone (nmol/l)	1.48 ± 0.15	2.55 ± 0.24	0.001
DHEAS (µmol/l)	6.47 ± 0.70	7.36 ± 0.47	0.124

\*Values are mean ± SEM. †Nonparametric Mann–Whitney *U*-test.

HOMA and/or CIGMA were less than 70%. HOMA %S significantly correlated to CIGMA %S in the PCOS patients with  $r = 0.82$  ( $P < 0.001$ ,  $n = 62$ ). Body mass index (BMI) was calculated as body weight (kg) divided by body height squared (m<sup>2</sup>). Waist-to-hip ratio (WHR) was calculated as waist divided by hip circumference.

Fasting blood samples of 35 healthy female subjects with no known medication, menstrual disorders or signs of hyperandrogenism were taken in the early follicular phase and served as healthy controls.

### Assays

Blood glucose was measured by the glucose dehydrogenase method (Hoffmann La Roche, Grenzach, Germany). Serum insulin was measured by a commercial radioimmunoassay (RIA) (Pharmacia, Freiburg, Germany) with a lower limit of sensitivity of < 14 pmol/l and intra- and interassay coefficients of variation (CVs) of 5.8% and 5.8%, respectively. Adiponectin was also determined by RIA (LincoResearch, St Charles, USA). The intra-assay CV ranged between 0.1 and 6.2% in various measurements in our laboratory and the interassay CV was 5.0%. Serum 17-OH-progesterone was determined by RIA (Schering, Berlin, Germany) with a lower level of sensitivity of 0.06 nmol/l and intra- and interassay CVs of 3.2% and 3.9%, respectively. Serum LH and progesterone were measured by chemiluminescence immunoassays using kits obtained from Bayer Diagnostics (Fernwald, Germany). The intra- and interassay CVs were, respectively, 2.9% and 2.4% for LH and 3.7% and 3.9% for progesterone, and the minimal detectable concentrations were 0.07 mU/ml (LH) and 0.48 nmol/l (progesterone). DHEAS was assayed by a chemiluminescence

immunoassay purchased from Nichols Institute Diagnostics (Bad Nauheim, Germany) with a lower sensitivity of 0.027 µmol/l and intra- and interassay CVs of 7.1% and 9.0%, respectively. Serum FSH was determined by an immunoradiometric assay (BioChem ImmunoSystems, Freiburg, Germany) with a lower sensitivity of 0.3 mU/ml and inter- and intra-assays CV of 2% and 3.1%, respectively. Oestradiol (E<sub>2</sub>), total testosterone (T) and SHBG were measured by RIA (obtained from DSL, Sinsheim, Germany) with lower detectable concentrations of 8.1 pmol/l, 0.28 nmol/l and 5 nmol/l. The respective inter- and intra-assay CVs were 2.2% and 2.7% for E<sub>2</sub>, 9.6% and 8.6% for T, and 2.2% and 4.4% for SHBG. Androstenedione was determined by RIA (Coulter Immunotech, Marseille, France) with a lower sensitivity of 0.14 nmol/l and inter- and intra-assay CVs of 5.6% and 6%, respectively.

### Body composition

Body fat mass was assessed using whole-body scans by dual-energy X-ray-absorptiometry (DEXA, Lunar, USA). Regions were positioned manually. The trunk was defined as follows: first, a horizontal line was placed across the shoulders to demarcate the head region; second, vertical lines were drawn through the shoulder joints, defining the arms; third, diagonal lines were placed through the hip joints, defining the lower limbs distal to this; and finally, the trunk was defined by excluding limb and head regions. CVs were determined by repeated measurements and were 2.2% for total and 7% for truncal fat mass.

### Statistics

Statistical analyses was performed with SPSS software (version 8.0, SPSS Inc., Chicago, IL, USA). Mean values are reported ± standard error of the mean (SEM). Significance was considered at two-tailed  $\alpha < 0.05$ . The nonparametric Mann–Whitney *U*-test was used to analyse for differences in skewed continuous variables, while differences in normally distributed continuous variables were compared by unpaired Student's *t*-test. Multiple comparisons were assessed by analysis of variance (ANOVA) followed by Bonferroni's test. A paired Wilcoxon test was performed to compare characteristics before and during metformin treatment. Spearman correlation coefficients were used to test the correlation between plasma adiponectin and clinical or biochemical parameters. To identify independent determinants of insulin resistance in women with PCOS, multiple linear regression analysis was performed after log-transformation of CIGMA and HOMA values to achieve a normal distribution.

### Results

The characteristics of the PCOS patients and the healthy controls are summarized in Table 1. In women with PCOS, testosterone,

**Table 2** Clinical and endocrine features of PCOS patients and healthy controls stratified by BMI

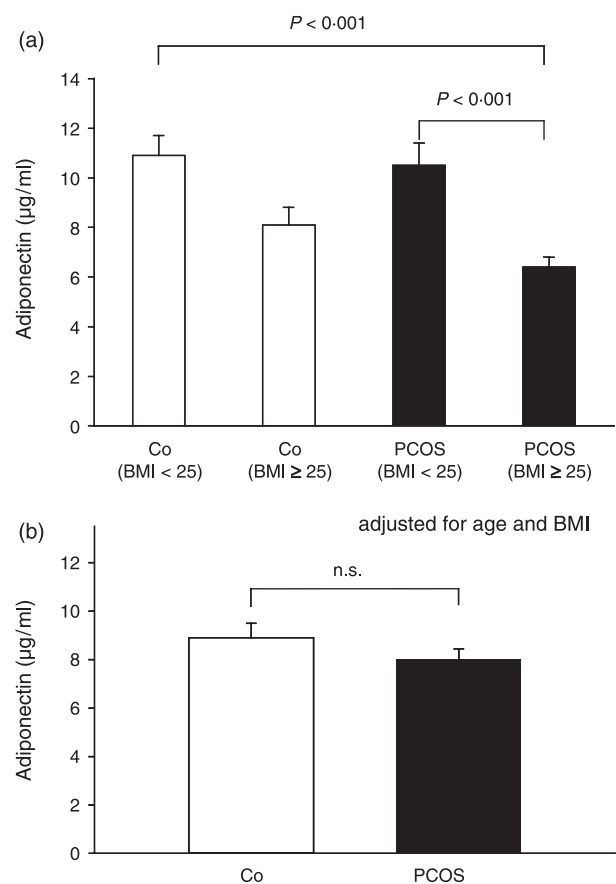
Variable	BMI < 25 kg/m <sup>2</sup>		BMI ≥ 25 kg/m <sup>2</sup>	
	Controls (n = 21)	PCOS (n = 17)	Controls (n = 14)	PCOS (n = 46)
Age (years)	28.7 ± 1.2	28.6 ± 1.4	32.8 ± 1.5	29 ± 0.7*
BMI (kg/m <sup>2</sup> )	21.6 ± 0.4	21.7 ± 0.5	31.1 ± 1.1	33.9 ± 1.0
WHR	0.75 ± 0.01	0.75 ± 0.01	0.82 ± 0.02	0.82 ± 0.01
HOMA (%S)	119 ± 7	130 ± 14	99 ± 11	69 ± 5*
Testosterone (nmol/l)	1.42 ± 0.17	2.53 ± 0.24**	2.08 ± 0.24	3.64 ± 0.21**
Androstenedione (nmol/l)	3.80 ± 0.24	7.88 ± 0.66***	4.85 ± 0.56	8.34 ± 0.42***
17-OH-Progesterone (nmol/l)	1.24 ± 0.06	2.33 ± 0.27*	1.61 ± 0.30	2.61 ± 0.27*
DHEAS (μmol/l)	6.39 ± 0.47	7.05 ± 0.91	6.47 ± 1.30	6.29 ± 0.55
LH/FSH	1.01 ± 0.09	1.79 ± 0.23*	0.97 ± 0.14	2.08 ± 0.39**

Mean ± SEM and *P*-values of nonparametric Mann–Whitney *U*-test are indicated.

\**P* < 0.05; \*\**P* < 0.01; \*\*\**P* < 0.001 (PCOS vs. control).

androstenedione, 17-OH-progesterone and the LH/FSH ratio were increased significantly and they had a higher BMI and were more insulin resistant according to fasting insulin concentrations and HOMA analysis compared with the healthy controls. Plasma adiponectin concentrations averaged to 7.6 ± 0.5 μg/ml (*n* = 62) in patients with PCOS, that is significantly lower than in controls (9.8 ± 0.6 μg/ml; *n* = 35; *P* = 0.006).

As plasma adiponectin levels are known to correlate with BMI, we stratified the control and PCOS women according to their degree of obesity (BMI < 25 kg/m<sup>2</sup> or ≥ 25 kg/m<sup>2</sup>) to obtain a homogeneous BMI distribution between the groups. The major characteristics of the groups are given in Table 2. The adiponectin concentrations were lower in the obese groups with BMI ≥ 25 kg/m<sup>2</sup> in both PCOS and control women (Fig. 1a). However, no significant differences were obtained between BMI-matched groups. In line with these data, an age- and BMI-adjusted analysis of plasma adiponectin levels did not reveal a significant difference between controls and women with PCOS (Fig. 1b). To define parameters associated with plasma levels of adiponectin we correlated adiponectin with BMI, fasting insulin, HOMA %S, testosterone, androstenedione, 17-OH-progesterone and the LH/FSH-ratio in the whole cohort (controls and PCOS women). There was a significant correlation of adiponectin with BMI (*r* = -0.516, *P* < 0.001), fasting insulin (*r* = -0.404, *P* < 0.001), HOMA %S (*r* = -0.424, *P* < 0.001) and testosterone (*r* = -0.279, *P* < 0.01), but not with androstenedione (*r* = -0.112, *P* = 0.325), 17-OH-progesterone (*r* = -0.031, *P* = 0.784) or the LH/FSH ratio (*r* = -0.033, *P* = 0.753). Similar results were obtained when the correlations were calculated separately in PCOS and in control women (data not shown). Multiple linear regression analysis was performed to further investigate whether obesity (BMI), insulin resistance (HOMA %S) and/or testosterone are independently



**Fig. 1** Adiponectin plasma levels in women with PCOS and in controls (a) after stratification for BMI and (b) after adjustment for age and BMI. Means ± SEM are presented.

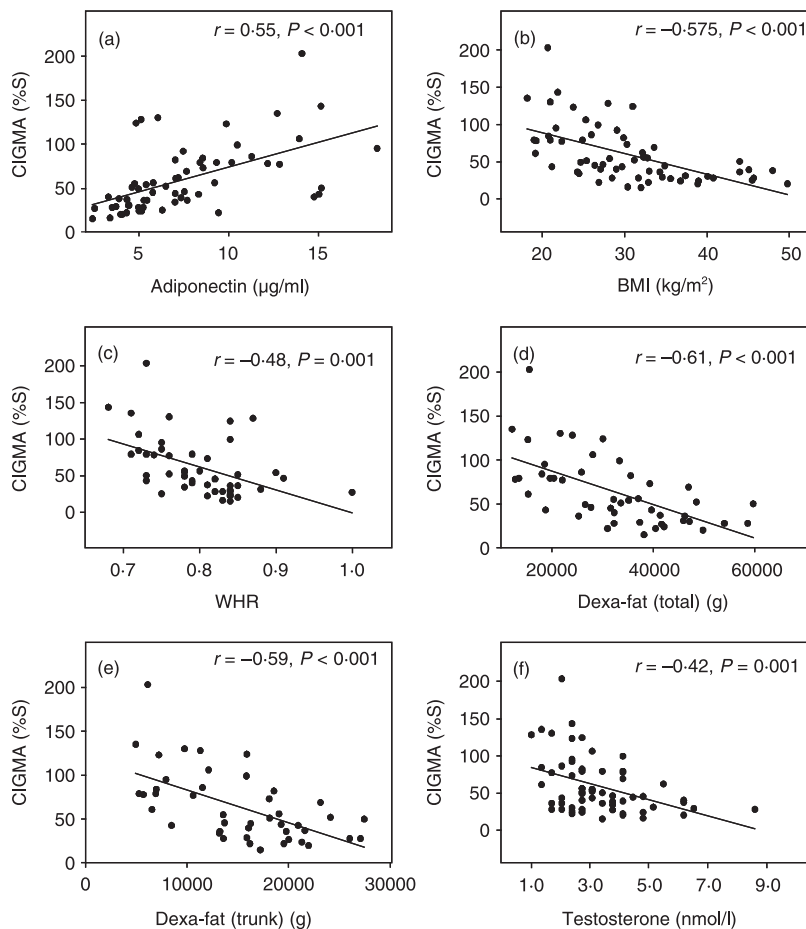
associated with circulating adiponectin concentrations. The model including all three parameters showed an  $R^2$  of 0.31. The beta coefficients were  $-0.315$  ( $P = 0.014$ ) for BMI,  $0.265$  ( $P = 0.043$ ) for HOMA %S, and  $-0.057$  ( $P = 0.612$ ) for testosterone demonstrating that BMI and insulin resistance, but not testosterone, are independently associated with adiponectin plasma levels. Taking into account the correlation between adiponectin and the respective parameters, BMI and HOMA %S accounted for 16.1% and 13.2% of the variability of plasma adiponectin, respectively. As the whole model accounted for only 31%, additional factors controlling circulating adiponectin must be assumed. These parameters, however, are different from the classical PCOS-associated endocrine factors such as hyperandrogenaemia. Consistent with this view, there was no association of plasma adiponectin with PCOS, when PCOS was used as a factor in the multiple linear regression analysis.

As adiponectin appears to be associated with insulin resistance and because insulin resistance has been proposed to aggravate PCOS-related clinical and endocrine alterations, we investigated the parameters that affect insulin sensitivity in women with

PCOS, and we tested whether circulating adiponectin could be involved.

Spearman correlations were calculated to define parameters associated with insulin sensitivity in PCOS patients. As depicted in Fig. 2 there was a highly significant positive correlation between CIGMA %S and plasma adiponectin ( $r = 0.55$ ;  $P < 0.001$ ), and there were inverse correlations of CIGMA %S with BMI ( $r = -0.575$ ;  $P < 0.001$ ), WHR ( $r = -0.48$ ;  $P = 0.001$ ), Dexa-fat (total) ( $r = -0.61$ ;  $P < 0.001$ ), Dexa-fat (trunk) ( $r = -0.59$ ;  $P < 0.001$ ) and testosterone levels ( $r = -0.42$ ;  $P = 0.001$ ). There were, however, no correlations of CIGMA %S with the LH/FSH ratio ( $r = -0.05$ ,  $P = 0.7$ ),  $E_2$  ( $r = -0.08$ ,  $P = 0.5$ ), progesterone ( $r = 0.05$ ,  $P = 0.6$ ), androstenedione ( $r = -0.09$ ,  $P = 0.5$ ), 17-OH-progesterone ( $r = -0.13$ ,  $P = 0.3$ ) or DHEAS ( $r = -0.1$ ,  $P = 0.4$ ). Comparable results were obtained in the whole cohort (PCOS patients and controls) using HOMA %S or fasting insulin as markers of insulin sensitivity (data not shown).

Multiple linear regression analysis was performed to further investigate the parameters with associated insulin resistance as



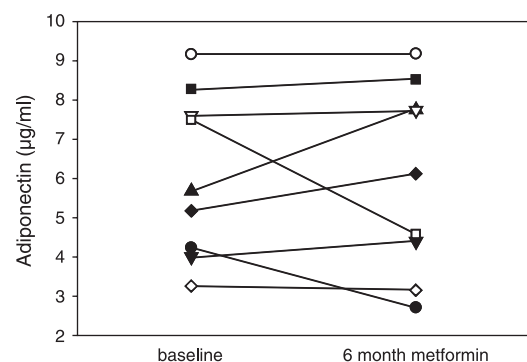
**Fig. 2** Insulin resistance (CIGMA %S) in women with PCOS in relation to (a) adiponectin, (b) BMI, (c) WHR, (d) Dexa-fat (total), (e) Dexa-fat (trunk) and (f) testosterone.

**Table 3** Linear regression analysis in women with PCOS using InCIGMA as the dependent variable and including BMI, Dexa-fat (total) for general obesity or Dexa-fat (trunk) representing abdominal obesity

	Standardized beta	Correlation	Standardized beta × correlation × 100 (%)	P-value
<b>BMI</b>				
Age	0.22	0.252	5.5	0.02
Adiponectin	0.321	0.586	18.8	0.003
BMI	-0.413	-0.61	25.2	< 0.001
Testosterone	-0.183	-0.414	7.5	0.058
R <sup>2</sup> of complete model			57	
<b>Dexa-fat (total) for general obesity</b>				
Age	0.273	0.301	8.2	0.011
Adiponectin	0.302	0.574	17.3	0.011
DEXA fat (total)	-0.462	-0.631	29.2	< 0.001
Testosterone	-0.116	-0.416	4.8	0.294
R <sup>2</sup> of complete model			59.5	
<b>Dexa-fat (trunk) representing abdominal obesity</b>				
Age	0.267	0.316	8.4	0.022
Adiponectin	0.314	0.562	17.7	0.014
DEXA fat (trunk)	-0.396	-0.589	23.3	< 0.001
Testosterone	-0.148	-0.442	6.5	0.222
R <sup>2</sup> of complete model			56	

indicated by CIGMA %S in PCOS and to test whether adiponectin is an independent factor here. As depicted in Table 3, age, various markers of obesity, such as BMI and total or truncal fat-mass, and adiponectin were independently associated with CIGMA %S. By contrast, testosterone was not a significant factor in the multiple regression analysis, suggesting that PCOS *per se* did not affect insulin sensitivity independent of obesity, age and adiponectin (Table 3). The models presented in Table 3 had adjusted R<sup>2</sup> values of 0.560–0.595, therefore explaining about 60% of the variability of insulin resistance in PCOS. According to these models about 18% of insulin resistance could be attributed to circulating plasma adiponectin (Table 3).

Nine obese and insulin-resistant PCOS women were treated with metformin. After metformin treatment for 6 months the women were less obese [body weight:  $-1.73 \pm 0.67$  kg,  $P = 0.011$ ], Dexa-fat (total):  $34\,376 \pm 1964$  g vs.  $39\,444 \pm 2542$  g,  $P = 0.028$ ; Dexa-fat (trunk):  $15\,782 \pm 888$  g vs.  $19\,012 \pm 988$  g,  $P = 0.028$ ; BMI:  $29.6 \pm 1.0$  kg/m<sup>2</sup> vs.  $31.6 \pm 1.25$  kg/m<sup>2</sup>,  $P = 0.025$ ] and hyperandrogenaemia had improved (testosterone:  $0.75 \pm 0.70$  pg/ml vs.  $1.05 \pm 0.13$  pg/ml,  $P = 0.032$ ). Insulin sensitivity increased, but the changes in HOMA %S and CIGMA %S failed to be significant [ $83 \pm 8\%$ (S) vs.  $69 \pm 9\%$ (S);  $P = 0.1$ , and  $48 \pm 4\%$ (S) vs.  $37 \pm 4\%$ (S);  $P = 0.12$ , respectively]. By contrast, adiponectin values did not change during the treatment period ( $6.1 \pm 0.8$  µg/ml vs.  $6.2 \pm 0.7$  µg/ml,  $P = 0.59$ ; Fig. 3). Given an alpha- and beta-error of 5%, power analysis indicated that by using the data of nine treated patients we were able to detect differences of 23% or more in plasma adiponectin.

**Fig. 3** Influence of metformin treatment on adiponectin plasma levels in nine women with PCOS.

## Discussion

In the present study we demonstrated that PCOS *per se* is not associated with low levels of adiponectin, which is in accordance with previous reports on PCOS women (Orio *et al.*, 2003; Panidis *et al.*, 2003). This suggests that adiponectin is not directly involved in the pathogenesis of PCOS. Both in women suffering from PCOS and in healthy controls plasma levels of the adipocytokine adiponectin were correlated with BMI, fasting insulin, HOMA %S and testosterone levels, but not with other markers of hyperandrogenaemia or with the LH/FSH ratio. Multiple linear regression analysis revealed that circulating adiponectin

concentrations were independently associated with the degree of obesity and insulin resistance as indicated by HOMA both in healthy controls and in PCOS women. By contrast, testosterone was not an independent factor associated with circulating plasma adiponectin despite indications that androgens could decrease circulating adiponectin levels (Arita *et al.*, 1999; Hotta *et al.*, 2000). BMI and HOMA %S accounted for about 16% and 13% of the variability of plasma adiponectin, respectively. As the linear model including BMI and HOMA %S accounted for only about 30% of the adiponectin levels, additional parameters controlling adiponectin levels must be assumed; these are, however, distinct from the classical PCOS-associated endocrine factors. Our findings extend those previously reported on PCOS women, which concluded that various measures of obesity such as total weight, waist circumference, BMI and variations in fat mass predominantly, if not exclusively, determine plasma adiponectin in PCOS (Orio *et al.*, 2003; Panidis *et al.*, 2003). This is in line with a number of recent reports from nondiabetic cohorts and type 2 diabetic patients that uniformly demonstrate a significant association of adiponectin with indicators of the insulin resistance syndrome, independent of increased adiposity (Weyer *et al.*, 2001; Pellme *et al.*, 2003; Shand *et al.*, 2003; Tschritter *et al.*, 2003).

Insulin resistance is common in PCOS and has been linked to PCOS-related clinical and endocrine alterations, such as hyperandrogenism and reproductive disorders (Robinson *et al.*, 1993; De Leo *et al.*, 2003). In addition, insulin resistance and the associated hyperinsulinaemia might be central to the high risk for impaired glucose tolerance and type 2 diabetes mellitus, as well as atherosclerosis and cardiovascular disease observed in PCOS women (Conway *et al.*, 1992; Guzick *et al.*, 1996; Birdsall *et al.*, 1997; Ehrmann *et al.*, 1999; Legro *et al.*, 1999; Lobo & Carmina, 2000). We therefore investigated parameters determining insulin sensitivity in PCOS and tested whether circulating adiponectin is involved. In our study, differences in insulin resistance between controls and women with PCOS were basically affected by age, obesity, testosterone and adiponectin levels. All other hormonal parameters investigated were not correlated with the degree of insulin resistance. Multiple linear regression analysis revealed that markers of obesity, such as BMI, total or truncal fat mass, age and adiponectin were independently associated with the degree of insulin resistance in PCOS women. By contrast, testosterone was not a significant and independent parameter, suggesting that PCOS *per se* is not a major factor in enhancing insulin resistance. Adiponectin accounted for about 18% of the variability of insulin resistance in PCOS independent of the degree of obesity, which accounted for 23–30%. This is in accordance with a previous report from a non-PCOS cohort demonstrating that plasma adiponectin is an obesity-independent predictor of the insulin resistance syndrome (Weyer *et al.*, 2001). Thus, although obesity is a driving force for circulating adiponectin concentrations, the

relationship between adiponectin and insulin sensitivity does not simply reflect obesity-associated insulin resistance. This is of particular interest with respect to reports indicating that insulin resistance in PCOS women is not entirely explained by the degree of obesity (Dunaif *et al.*, 1989; Ducluzeau *et al.*, 2003) and that additional factors are involved. Thus, adiponectin might be such a factor, which contributes to the obesity-independent development of insulin resistance in women with PCOS.

Adiponectin is exclusively and abundantly expressed in white adipose tissue, and is the most abundant circulating adipose-specific protein in humans known so far (Arita *et al.*, 1999). *In vitro* and *in vivo* studies in rodents have shown that adiponectin has the potential to enhance insulin sensitivity and to improve glucose metabolism (Weyer *et al.*, 2001; Yamauchi *et al.*, 2001; Kubota *et al.*, 2002; Maeda *et al.*, 2002). The precise mechanisms by which adiponectin ameliorates insulin resistance and glucose metabolism are currently under investigation, although it is known that the insulin-sensitizing action involves both the liver and muscle (Berg *et al.*, 2001; Yamauchi *et al.*, 2001). Besides its effects on insulin sensitivity and glucose metabolism, adiponectin may have an additional antiatherogenic potential as adiponectin<sup>-/-</sup> mice showed two times more neointimal formation in response to external vascular cuff injury compared to wild-type mice (Kubota *et al.*, 2002). Consistent with a similar role in humans, plasma adiponectin concentrations were found to be decreased in individuals with obesity, type 2 diabetes mellitus, and cardiovascular disease (Ouchi *et al.*, 1999; Hotta *et al.*, 2000; Weyer *et al.*, 2001). In addition, hypoadiponectinaemia has been shown to precede the onset of type 2 diabetes, again supporting its involvement in the development of disease (Lindsay *et al.*, 2002; Spranger *et al.*, 2003a). Thus, circulating adiponectin may not only determine the degree of insulin resistance but could also provide a link to the high risk for type 2 diabetes mellitus and cardiovascular disease in PCOS patients with low circulating adiponectin.

Metformin is currently in widespread clinical use for treatment of PCOS-related symptoms (De Leo *et al.*, 2003). Based on surrogate parameters several reports have suggested improvement in the risk for the development of type 2 diabetes and cardiovascular disease by metformin (De Leo *et al.*, 2003). In our patients metformin treatment resulted in a significant weight loss and reduction in total fat mass and caused a decrease in total testosterone levels, as reported (Harborne *et al.*, 2003). Circulating adiponectin, however, did not change over a 6-month treatment period. Again, this does not support the view that adiponectin levels in PCOS are directly and predominantly determined by BMI or fat mass (Orio *et al.*, 2003; Panidis *et al.*, 2003). It is, however, consistent with an obesity-independent association of plasma adiponectin with the degree of insulin resistance in PCOS, as changes in HOMA %S or CIGMA &S were not significant under metformin therapy. Furthermore, as in type 2

diabetics (Phillips *et al.*, 2003), metformin treatment does not appear to exert additional or favourable effects on plasma adiponectin in PCOS. However, our results on metformin and adiponectin are based on a noncontrolled study with a small number of participants and needs to be confirmed in larger cohorts.

In summary, in PCOS women plasma adiponectin is independently associated with markers of obesity and insulin resistance. Furthermore, circulating adiponectin is an independent factor associated with the degree of insulin resistance in PCOS. Our observations, combined with emerging functional evidence, support the concept that low levels of adiponectin may contribute to the development and/or maintenance of insulin resistance and may provide a link to the risk of type 2 diabetes mellitus and cardiovascular disease in PCOS patients.

### Acknowledgements

We thank Katrin Sprengel, Stefanie Nedel and Bärbel Thon for excellent technical assistance. This project was funded by the German Diabetes Association (to J.S.).

### References

- Alessi, M.C., Peiretti, F., Morange, P., Henry, M., Nalbone, G. & Juhan-Vague, I. (1997) Production of plasminogen activator inhibitor 1 by human adipose tissue: possible link between visceral fat accumulation and vascular disease. *Diabetes*, **46**, 860–867.
- Arita, Y., Kihara, S., Ouchi, N., Takahashi, M., Maeda, K., Miyagawa, J., Hotta, K., Shimomura, I., Nakamura, T., Miyaoka, K., Kuriyama, H., Nishida, M., Yamashita, S., Okubo, K., Matsubara, K., Muraguchi, M., Ohmoto, Y., Funahashi, T. & Matsuzawa, Y. (1999) Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochemical and Biophysical Research Communications*, **257**, 79–83.
- Berg, A.H., Combs, T.P. & Scherer, P.E. (2002) ACRP30/adiponectin: an adipokine regulating glucose and lipid metabolism. *Trends in Endocrinology and Metabolism*, **13**, 84–89.
- Berg, A.H., Du Combs, T.P.X., Brownlee, M. & Scherer, P.E. (2001) The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nature Medicine*, **7**, 947–953.
- Birdsall, M.A., Farquhar, C.M. & White, H.D. (1997) Association between polycystic ovaries and extent of coronary artery disease in women having cardiac catheterization. *Annals of Internal Medicine*, **126**, 32–35.
- Combs, T.P., Berg, A.H., Obici, S., Scherer, P.E. & Rossetti, L. (2001) Endogenous glucose production is inhibited by the adipose-derived protein Acrp30. *Journal of Clinical Investigation*, **108**, 1875–1881.
- Conway, G.S., Agrawal, R., Betteridge, D.J. & Jacobs, H.S. (1992) Risk factors for coronary artery disease in lean and obese women with the polycystic ovary syndrome. *Clinical Endocrinology (Oxford)*, **37**, 119–125.
- Costello, M.F. & Eden, J.A. (2003) A systematic review of the reproductive system effects of metformin in patients with polycystic ovary syndrome. *Fertility and Sterility*, **79**, 1–13.
- De Leo, V., La Marca, A. & Petraglia, F. (2003) Insulin-lowering agents in the management of polycystic ovary syndrome. *Endocrine Reviews*, **24**, 633–667.
- Ducluzeau, P.H., Cousin, P., Malvoisin, E., Borne, H., Vidal, H., Laville, M. & Pugeat, M. (2003) Glucose-to-insulin ratio rather than sex hormone-binding globulin and adiponectin levels is the best predictor of insulin resistance in nonobese women with polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism*, **88**, 3626–3631.
- Dunaif, A. (1997) Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis. *Endocrine Reviews*, **18**, 774–800.
- Dunaif, A., Segal, K.R., Futterweit, W. & Dobrjansky, A. (1989) Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. *Diabetes*, **38**, 1165–1174.
- Ehrmann, D.A., Barnes, R.B., Rosenfield, R.L., Cavaghan, M.K. & Imperial, J. (1999) Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. *Diabetes Care*, **22**, 141–146.
- Festa, A., D'Agostino, R. Jr, Howard, G., Mykkanen, L., Tracy, R.P. & Haffner, S.M. (2000) Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS). *Circulation*, **102**, 42–47.
- Franks, S. (1995) Polycystic ovary syndrome. *New England Journal of Medicine*, **333**, 853–861.
- Freeman, D.J., Norrie, J., Caslake, M.J., Gaw, A., Ford, I., Lowe, G.D., O'Reilly, D.S., Packard, C.J. & Sattar, N. (2002) C-reactive protein is an independent predictor of risk for the development of diabetes in the West of Scotland Coronary Prevention Study. *Diabetes*, **51**, 1596–1600.
- Guzick, D.S., Talbot, E.O., Sutton-Tyrrell, K., Herzog, H.C., Kuller, L.H. & Wolfson, S.K. Jr. (1996) Carotid atherosclerosis in women with polycystic ovary syndrome: initial results from a case-control study. *American Journal of Obstetrics and Gynecology*, **174**, 1224–1232.
- Haas, D.A., Carr, B.R. & Attia, G.R. (2003) Effects of metformin on body mass index, menstrual cyclicity, and ovulation induction in women with polycystic ovary syndrome. *Fertility and Sterility*, **79**, 469–481.
- Harborne, L., Fleming, R., Lyall, H., Norman, J. & Sattar, N. (2003) Descriptive review of the evidence for the use of metformin in polycystic ovary syndrome. *Lancet*, **361**, 1894–1901.
- Hermans, M.P., Levy, J.C., Morris, R.J. & Turner, R.C. (1999) Comparison of insulin sensitivity tests across a range of glucose tolerance from normal to diabetes. *Diabetologia*, **42**, 678–687.
- Hotamisligil, G.S. & Spiegelman, B.M. (1994) Tumor necrosis factor alpha: a key component of the obesity-diabetes link. *Diabetes*, **43**, 1271–1278.
- Hotta, K., Funahashi, T., Arita, Y., Takahashi, M., Matsuda, M., Okamoto, Y., Iwahashi, H., Kuriyama, H., Ouchi, N., Maeda, K., Nishida, M., Kihara, S., Sakai, N., Nakajima, T., Hasegawa, K., Muraguchi, M., Ohmoto, Y., Nakamura, T., Yamashita, S., Hanafusa, T. & Matsuzawa, Y. (2000) Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arteriosclerosis, Thrombosis, and Vascular Biology*, **20**, 1595–1599.
- Hu, E., Liang, P. & Spiegelman, B.M. (1996) AdipoQ is a novel adipose-specific gene dysregulated in obesity. *Journal of Biological Chemistry*, **271**, 10697–10703.
- Kubota, N., Terauchi, Y., Yamauchi, T., Kubota, T., Moroi, M., Matsui, J., Eto, K., Yamashita, T., Kamon, J., Satoh, H., Yano, W., Nagai, R., Kimura, S., Kadowaki, T. & Noda, T. (2002) Disruption of adiponectin causes insulin resistance and neointimal formation. *Journal of Biological Chemistry*, **24**, 24.
- Legro, R.S., Kunselman, A.R., Dodson, W.C. & Dunaif, A. (1999) Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a prospective,

- controlled study in 254 affected women. *Journal of Clinical Endocrinology and Metabolism*, **84**, 165–169.
- Levy, J.C., Matthews, D.R. & Hermans, M.P. (1998) Correct homeostasis model assessment (HOMA) evaluation uses the computer program. *Diabetes Care*, **21**, 2191–2192.
- Lindsay, R.S., Funahashi, T., Hanson, R.L., Matsuzawa, Y., Tanaka, S., Tataranni, P.A., Knowler, W.C. & Krakoff, J. (2002) Adiponectin and development of type 2 diabetes in the Pima Indian population. *Lancet*, **360**, 57–58.
- Lobo, R.A. & Carmina, E. (2000) The importance of diagnosing the polycystic ovary syndrome. *Annals of Internal Medicine*, **132**, 989–993.
- Maeda, K., Okubo, K., Shimomura, I., Funahashi, T., Matsuzawa, Y. & Matsubara, K. (1996) cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose Most abundant Gene transcript 1). *Biochemical and Biophysical Research Communications*, **221**, 286–289.
- Maeda, N., Shimomura, I., Kishida, K., Nishizawa, H., Matsuda, M., Nagaretani, H., Furuyama, N., Kondo, H., Takahashi, M., Arita, Y., Komuro, R., Ouchi, N., Kihara, S., Tochino, Y., Okutomi, K., Horie, M., Takeda, S., Aoyama, T., Funahashi, T. & Matsuzawa, Y. (2002) Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. *Nature Medicine*, **17**, 17.
- Nakano, Y., Tobe, T., Choi-Miura, N.H., Mazda, T. & Tomita, M. (1996) Isolation and characterization of GBP28, a novel gelatin-binding protein purified from human plasma. *Journal of Biochemistry (Tokyo)*, **120**, 803–812.
- Nishizawa, H., Shimomura, I., Kishida, K., Maeda, N., Kuriyama, H., Nagaretani, H., Matsuda, M., Kondo, H., Furuyama, N., Kihara, S., Nakamura, T., Tochino, Y., Funahashi, T. & Matsuzawa, Y. (2002) Androgens decrease plasma adiponectin, an insulin-sensitizing adipocyte-derived protein. *Diabetes*, **51**, 2734–2741.
- Orio, F. Jr, Palomba, S., Cascella, T., Milan, G., Mioni, R., Pagano, C., Zullo, F., Colao, A., Lombardi, G. & Vettor, R. (2003) Adiponectin levels in women with polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism*, **88**, 2619–2623.
- Ouchi, N., Kihara, S., Arita, Y., Maeda, K., Kuriyama, H., Okamoto, Y., Hotta, K., Nishida, M., Takahashi, M., Nakamura, T., Yamashita, S., Funahashi, T. & Matsuzawa, Y. (1999) Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. *Circulation*, **100**, 2473–2476.
- Ouchi, N., Kihara, S., Arita, Y., Okamoto, Y., Maeda, K., Kuriyama, H., Hotta, K., Nishida, M., Takahashi, M., Muraguchi, M., Ohmoto, Y., Nakamura, T., Yamashita, S., Funahashi, T. & Matsuzawa, Y. (2000) Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF- $\kappa$ B signaling through a cAMP-dependent pathway. *Circulation*, **102**, 1296–1301.
- Panidis, D., Kourtis, A., Farmakiotis, D., Mouslech, T., Rouso, D. & Koliakos, G. (2003) Serum adiponectin levels in women with polycystic ovary syndrome. *Human Reproduction*, **18**, 1790–1796.
- Pasquali, R. & Gamberini, A. (2002) Treatment of the polycystic ovary syndrome with lifestyle intervention. *Current Opinion in Endocrinology and Diabetes*, **9**, 459–468.
- Pellme, F., Smith, U., Funahashi, T., Matsuzawa, Y., Brekke, H., Wiklund, O., Taskinen, M.R. & Jansson, P.A. (2003) Circulating adiponectin levels are reduced in nonobese but insulin-resistant first-degree relatives of type 2 diabetic patients. *Diabetes*, **52**, 1182–1186.
- Phillips, S.A., Ciaraldi, T.P., Kong, A.P., Bandukwala, R., Aroda, V., Carter, L., Baxi, S., Mudaliar, S.R. & Henry, R.R. (2003) Modulation of circulating and adipose tissue adiponectin levels by antidiabetic therapy. *Diabetes*, **52**, 667–674.
- Pradhan, A.D., Manson, J.E., Rifai, N., Buring, J.E. & Ridker, P.M. (2001) C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *Journal of the American Medical Association*, **286**, 327–334.
- Robinson, S., Kiddy, D., Gelding, S.V., Willis, D., Nithyanathan, R., Bush, A., Johnston, D.G. & Franks, S. (1993) The relationship of insulin insensitivity to menstrual pattern in women with hyperandrogenism and polycystic ovaries. *Clinical Endocrinology*, **39**, 351–355.
- Scherer, P.E., Williams, S., Fogliano, M., Baldini, G. & Lodish, H.F. (1995) A novel serum protein similar to C1q, produced exclusively in adipocytes. *Journal of Biological Chemistry*, **270**, 26746–26749.
- Shand, B.L., Scott, R.S., Elder, P.A. & George, P.M. (2003) Plasma adiponectin in overweight, nondiabetic individuals with or without insulin resistance. *Diabetes, Obesity and Metabolism*, **5**, 349–353.
- Shapiro, L. & Scherer, P.E. (1998) The crystal structure of a complement-1q family protein suggests an evolutionary link to tumor necrosis factor. *Current Biology*, **8**, 335–338.
- Spranger, J., Kroke, A., Mohlig, M., Bergmann, M.M., Ristow, M., Boeing, H. & Pfeiffer, A.F. (2003a) Adiponectin and protection against type 2 diabetes mellitus. *Lancet*, **361**, 226–228.
- Spranger, J., Kroke, A., Mohlig, M., Hoffmann, K., Bergmann, M.M., Ristow, M., Boeing, H. & Pfeiffer, A.F. (2003b) Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC) Potsdam Study. *Diabetes*, **52**, 812–817.
- Tschritter, O., Fritsche, A., Thamer, C., Haap, M., Shirkavand, F., Rahe, S., Staiger, H., Maerker, E., Häring, H. & Stumvoll, M. (2003) Plasma adiponectin concentrations predict insulin sensitivity of both glucose and lipid metabolism. *Diabetes*, **52**, 239–243.
- Warram, J.H., Kopczynski, J., Janka, H.U. & Krolewski, A.S. (1997) Epidemiology of non-insulin-dependent diabetes mellitus and its macrovascular complications. A basis for the development of cost-effective programs. *Endocrinology and Metabolism Clinics of North America*, **26**, 165–188.
- Weyer, C., Funahashi, T., Tanaka, S., Hotta, K., Matsuzawa, Y., Pratley, R.E. & Tataranni, P.A. (2001) Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *Journal of Clinical Endocrinology and Metabolism*, **86**, 1930–1935.
- Yamamoto, Y., Hirose, H., Saito, I., Tomita, M., Taniyama, M., Matsubara, K., Okazaki, Y., Ishii, T., Nishikai, K. & Saruta, T. (2002) Correlation of the adipocyte-derived protein adiponectin with insulin resistance index and serum high-density lipoprotein-cholesterol, independent of body mass index, in the Japanese population. *Clinical Science (London)*, **103**, 137–142.
- Yamauchi, T., Kamon, J., Waki, H., Terauchi, Y., Kubota, N., Hara, K., Mori, Y., Ide, T., Murakami, K., Tsuboyama-Kasaoka, N., Ezaki, O., Akanuma, Y., Gavrilova, O., Vinson, C., Reitman, M.L., Kagechika, H., Shudo, K., Yoda, M., Nakano, Y., Tobe, K., Nagai, R., Kimura, S., Tomita, M., Froguel, P. & Kadowaki, T. (2001) The fat-derived hormone adiponectin reverses insulin resistance associated with both lipotrophy and obesity. *Nature Medicine*, **7**, 941–946.
- Yudkin, J.S., Kumari, M., Humphries, S.E. & Mohamed-Ali, V. (2000) Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? *Atherosclerosis*, **148**, 209–214.
- Zawadzki, J.K. & Dunaif, A. (1992) Diagnostic criteria for polycystic ovary syndrome: towards a rational approach. In: *Current Issues in Endocrinology and Metabolism: Polycystic Ovary Syndrome* (eds A. Dunaif, J. Givens, F. Haseltine & G. Merriam), pp. 377–384. Blackwell, New York.