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CLINICAL STUDY

Adipocyte fatty acid-binding protein is associated with markers of obesity, but is an unlikely link between obesity, insulin resistance, and hyperandrogenism in polycystic ovary syndrome women

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Abstract

Objective: Many polycystic ovary syndrome (PCOS) women suffer from adiposity and insulin resistance (IR), which play an important role in the development and maintenance of PCOS. Adipocyte fatty acid-binding protein (A-FABP) is mainly expressed in adipocytes, and circulating A-FABP has been associated with markers of obesity and IR. Thus, as observed with other adipose tissue derived factors, secreted A-FABP might be involved in the pathogenesis of obesity-associated disorders such as PCOS.
Design: Plasma A-FABP concentrations were measured in 102 non-diabetic PCOS women, and associations with markers of obesity, IR, inflammation, and hyperandrogenism were investigated by correlation and multiple linear regression analyses. The effect of lifestyle intervention on A-FABP was studied in a second cohort of 17 obese PCOS women.
Results: A-FABP correlated with body mass index (BMI; $R=0.694$, $P<0.001$), dual-energy X-ray-absorptiometry (DEXA) fat mass ($R=0.729$, $P<0.001$), DEXA lean body mass ($R=0.399$, $P=0.001$), HOMA %S ($R=-0.435$, $P<0.001$), hsCRP ($R=0.355$, $P=0.001$), and free testosterone (fT; $R=0.230$, $P=0.02$). Adjusted for age, smoking, and glucose metabolism the association of A-FABP with HOMA %S was still significant ($P<0.001$), whereas the associations with fT ($P=0.09$) and hsCRP ($P=0.25$) were not. Inclusion of BMI into the model abolished the impact of A-FABP on HOMA %S. In BMI-matched PCOS women ($n=20$ pairs), neither HOMA %S ($P=0.3$) nor fT ($P=0.6$) were different despite different A-FABP levels ($P<0.001$), and in 17 obese PCOS women undergoing a lifestyle intervention, changes in IR were not paralleled by changes in A-FABP.
Conclusions: Circulating A-FABP was correlated with markers of obesity, but had no major impact on IR, inflammation, or hyperandrogenemia in PCOS women.

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Introduction

The polycystic ovary syndrome (PCOS) is one of the most frequent endocrine diseases in women (1, 2). Clinically it is characterized by hyperandrogenism, chronic anovulation, and infertility. Many PCOS women are overweight or obese, and suffer from insulin resistance (IR) and other features of the metabolic syndrome, which increase the risk for the development of type 2 diabetes and cardiovascular disease (3–7). PCOS is a heterogeneous disorder and its etiology appears to be complex and multifactorial. There is evidence that adiposity and associated metabolic alterations such as IR play an important role in the development and maintenance of PCOS pathology at least in a substantial proportion of patients (3, 4, 6, 7). There is a close correlation between adiposity, the degree of IR, and the severity of hyperandrogenism. Weight reduction and amelioration of metabolic abnormalities, especially those related to IR, by lifestyle or pharmacological intervention have been shown to improve hyperandrogenism, menstrual regularity, and fertility (7–12). However, the mechanisms that link adiposity, IR, and features of PCOS such as hyperandrogenism are only incompletely understood. Obesity, characterized by excess accumulation of adipose tissue, is a known risk factor for IR, and hyperinsulinemia associated with the insulin resistant state appears to enhance hyperandrogenism and anovulation through a number of mechanisms (7, 13, 14). In addition, altered

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sex steroid metabolism by adipocytes may also contribute and form a more direct link between obesity and PCOS features (7, 15).

Adipocytes are significantly involved in lipid metabolism. Intracellular lipid metabolism is regulated in part by the cytosolic fatty acid-binding proteins (FABP). FABPs are a family of proteins which are expressed in a tissue-specific manner and that are involved in shuttling fatty acids to cellular compartments, modulating intracellular lipid metabolism, and regulating gene expression (16). One of these is the adipocyte FABP (A-FABP). A-FABP has been shown to affect insulin sensitivity, lipid metabolism, and inflammatory responses associated with atherosclerosis (16–18). Mice deficient in A-FABP exhibit lower obesity-related IR and protection against atherosclerosis in models of hypercholesterolemia (19–21). In humans, a genetic variant that reduces the promoter activity of the *A-FABP* gene appears to reduce the risk for hypertriglyceridemia, type 2 diabetes, and cardiovascular disease (22). Although *A-FABP* is predominantly expressed in the cytosol of mature adipocytes, it is also secreted by human adipocytes into the bloodstream. Circulating A-FABP has been recently described to be associated with markers of obesity and IR (23, 24). Thus, the possibility exists that secreted A-FABP like other adipose tissue-derived bioactive molecules (e.g., tumor necrosis factor-α, interleukin-6, or adiponectin) is involved in the pathogenesis of obesity-associated pathologies such as IR, type 2 diabetes, cardiovascular disease, and also PCOS.

In the present study, we investigated a potential role for circulating A-FABP in IR, subclinical inflammation, and endocrine abnormalities (hyperandrogenemia) in women with PCOS.

Subjects and methods

Subjects

A cohort of 102 consecutive non-diabetic PCOS women, who were referred to our clinic because of hirsutism or oligo-/amenorrhea, were included after written informed consent was obtained. The cohort has been studied in part previously (25–27). The diagnosis of PCOS was based on a) the presence of cycle abnormalities i.e. oligomenorrhea (four or fewer cycles in the last six months) or amenorrhea (no cycles in the last six months) and b) clinical signs of hyperandrogenism i.e. hirsutism, defined as a Ferriman–Gallwey Score ≥ 8 (28) or c) laboratory findings i.e. hyperandrogenemia, defined as serum androgen levels (total testosterone, androstenedione, or DHEAS) above the upper limit of normal for the respective assay, and d) the exclusion of other disorders such as Cushing's syndrome (by a low-dose (1 mg) overnight dexamethasone suppression test), late-onset 21-hydroxylase deficiency (by an adrenocorticotropin (ACTH)-stimulation test and genetic testing in case of a suspicious test result), thyroid dysfunction, hyperprolactinemia, or androgen-secreting tumors. These diagnostic criteria for PCOS are consistent with the most commonly used diagnostic criteria for PCOS, often referred to as the National Institutes of Health (NIH) consensus criteria (29). The clinical and endocrine features of the women are given in Table 1. Eight patients were euthyroid under thyroid hormone replacement because of Hashimoto's thyroiditis. Five women had arterial hypertension and were normotensive under antihypertensive drugs. Five women suffered from asthma, allergic rhinitis, or hyperkinetic heart syndrome. Eighty-five women were not taking any medication.

All women were studied within the first 10 days following menstruation in case of mild oligomenorrhea, or at random if they suffered severe oligo- or -amenorrhea. Blood was sampled in the morning after an overnight fast and the samples were stored at -20 °C until analysis. An oral glucose tolerance test was performed in all women to define glucose metabolism. Women with diabetes according to the World Health Organization definition (30) were excluded from the analysis because of the endpoint IR. Amongst the 102 women, 85 women had normal glucose tolerance (NGT) and 17 women suffered from impaired glucose tolerance (IGT). IR was quantified by calculating HOMA %S using the mean of three fasting glucose and insulin values (31) and the HOMA2 program which was kindly provided by Dr Levy (32). In 99 women, IR was additionally assessed by a 2-h continuous infusion of glucose with model assessment (CIGMA) again using the HOMA2 program.

A second cohort of obese PCOS women (age 31.2 ± 1.33 years, body mass index (BMI) $= 39.4 \pm 1.47$ kg/m^2, $n=17$) was studied at baseline and after

Table 1 Clinical and endocrine features of the PCOS cohort ($n=102$). Continuous variables are given as mean$\pm$S.E.M. and frequencies as *n* (%).

Characteristic	
Age (years)	28.93$\pm$0.51
BMI (kg/m^2)	31.50$\pm$0.78
Fasting plasma glucose (mmol/l)	4.60$\pm$0.06
Fasting insulin (pmol/l)	97.78$\pm$6.40
HOMA %S	78.29$\pm$4.61
hsCRP (mg/l)	3.79$\pm$0.55
Total testosterone (nmol/l)	3.19$\pm$0.12
Calculated free testosterone (pmol/l)	57.39$\pm$3.66
Estradiol (pmol/l)	251.6$\pm$29.6
Progesterone (nmol/l)	4.82$\pm$1.05
LH (U/l)	8.55$\pm$0.48
LH/FSH	1.70$\pm$0.11
DHEAS (nmol/l)	7.58$\pm$0.38
Androstenedione (nmol/l)	8.22$\pm$0.31
SHBG (nmol/l)	52.55$\pm$4.41
17-OH-progesterone (nmol/l)	2.37$\pm$0.15
DEXA-total fat mass (kg)	34.17$\pm$1.49
DEXA-lean body mass (kg)	45.00$\pm$0.78
Overweight/obese subjects (BMI >25 kg/m^2)	77 (75.5%)
Smokers	7 (5.9%)

16 weeks of lifestyle intervention. All the women had a) cycle abnormalities i.e. oligomenorrhea (four or fewer cycles in the last six months) or amenorrhea (no cycles in the last four months) and b) hirsutism, defined as a Ferriman–Gallwey score ≥ 8 (28) or c) hyperandrogenemia, defined as total testosterone above the upper limit of normal, and d) the exclusion of other disorders as described above. Thus, all PCOS women of this second cohort fulfilled the NIH consensus criteria (29). The lifestyle modification consisted of a comprehensive individual counseling on nutrition and physical activity, monthly meetings in a group setting, and weekly participation in aqua gymnastics classes. Based on the individual report dietary recommendations were given. HOMA %S was calculated using fasting glucose and insulin from one blood sample.

Assessment of body composition

BMI was calculated as body weight (kg) divided by body height squared (m^2). Body fat mass and lean body mass were assessed in a subgroup of 70 women using whole-body scans by DEXA (Lunar, Madison, WI, USA). Coefficient of variance was determined by repeated measurements and was 2.2% for total fat mass and 1% for lean body mass.

Assays

All biochemical and endocrine parameters were determined as previously described (26, 33). Total testosterone (DSL, Sinsheim, Germany) and androstenedione (Couter Immunotech, Marseille, France) were measured by RIA, and DHEAS was quantified using a chemiluminescence immunoassay (Nichols, Bad Nauheim, Germany). The upper limits of normal according to the manufacturer's information were 2.8 nmol/l, 12.0 µmol/l, and 10.5 nmol/l for total testosterone, DHEAS, and androstenedione respectively. Free testosterone was calculated from total testosterone and sex hormone-binding globulin (SHBG) as published (34) using a web-based calculator (http://www.issam.ch/freetesto.htm). In the second cohort of 17 obese PCOS women, total testosterone was measured by a competitive immunoassay (ADVIA Centaur, Bayer Diagnostics). An upper limit of normal (2.6 nmol/l) was used according to the manufacturer's information.

Plasma A-FABP was measured in duplicate using a commercially available ELISA (BioVendor, Heidelberg, Germany). The intra- and interassay coefficients of variation were 3.9 and 5.1% respectively. High sensitive C-reactive protein (hsCRP) was measured in a subgroup of 82 women with an immunoturbidimetric assay (Wako, Neuss, Germany) on Cobas Mira laboratory system.

Statistical analysis

Statistical analyses were performed with SPSS software (version 8.0, SPSS Inc., Chicago, IL, USA). Mean values are reported $\pm$standard error of the mean (S.E.M.). Differences were considered significant when two-tailed $\alpha < 0.05$. Correlation analyses were performed using the Spearman correlation coefficient. For the comparison, however, of the two regression matrices (A-FABP and DEXA-total fat mass, A-FABP and DEXA-lean body mass) *z*-transformation (35) was performed using Pearson's correlation coefficients. Prior to the calculation of the Pearson correlation, A-FABP had to be ln transformed to achieve normal distribution, while DEXA-fat mass and DEXA-lean body mass were normally distributed variables as tested by Kolmogorov–Smirnov. The relations between A-FABP and BMI, hsCRP, and free testosterone were addressed by multiple linear regression analyses. In the BMI-matched subgroups and the lifestyle intervention cohort, the parameters were compared by the Student's *t*-test for paired analysis after ln transformation to achieve a normal distribution. Age could not be normally distributed and was compared by the non-parametric Wilcoxon test.

Results

The clinical and endocrine characteristics of the cohort are given in Table 1. A-FABP ranged from 5.1 to 113.0 ng/ml with a mean value of 29.6 ± 1.9 ng/ml and a median of 23.8 ng/ml ($n = 102$). There was a significant correlation between A-FABP and BMI and between A-FABP and DEXA-total fat mass or DEXA-lean body mass, which were assessed in a subgroup of 70 patients Table 2). The correlation of A-FABP with DEXA-total fat mass was significantly better than the correlation of A-FABP with DEXA-lean body mass ($P < 0.05$), which is consistent with the concept that A-FABP is released from the adipose tissue and is closely associated with the degree of adiposity.

In PCOS women, obesity is tightly associated with IR, subclinical inflammation, and hyperandrogenemia (7, 9, 26, 36–40). We therefore investigated the relation between circulating A-FABP and HOMA %S as a

Table 2 Correlation between adipocyte fatty acid-binding protein and body mass index (BMI), dual-energy X-ray-absorptiometry (DEXA)-total fat mass, DEXA-lean body mass, HOMA %S, free testosterone, and high sensitive C-reactive protein (hsCRP).

	Correlation coefficient *R*	*P*-value
BMI (kg/m^2)	0.694	<0.001
DEXA-total fat mass (kg)	0.729	<0.001
DEXA-lean body mass (kg)	0.399	0.001
HOMA %S (%)	−0.435	<0.001
Free testosterone (pmol/l)	0.23	0.02
hsCRP	0.355	0.001

measure of IR, hsCRP as a marker of inflammatory activity, and free testosterone. A-FABP was significantly correlated with all the three parameters Table 2). To further explore the impact of A-FABP on these parameters, multiple linear regression analyses were performed with HOMA %S, BMI, or free testosterone as the dependent variable. Adjusted for putative confounders such as age, smoking, and the state of glucose metabolism (NGT versus IGT) A-FABP was significantly associated with HOMA %S (β-coefficient -0.367, $P<0.001$), while there were no significant associations with free testosterone ($P=0.09$) or hsCRP ($P=0.25$). The use of total testosterone instead of calculated free testosterone revealed a similar result ($P=0.64$). Further inclusion into the model of BMI, which by itself was significantly associated with HOMA %S (β-coefficient -0.558, P-value <0.001) completely abolished the relationship between A-FABP and HOMA %S (P-value $=0.8$). This result was confirmed by using CIGMA %S as a second measure of IR (data not shown).

The fact that adding BMI into the adjusted model eliminated the association between A-FABP and HOMA %S could either indicate that circulating A-FABP is just a circulating biomarker of adiposity without any independent effect on HOMA %S or that it is an important molecular link between adiposity and IR in hyperandrogenemic PCOS women. In the latter case, it could be expected that in BMI-matched PCOS women differences in A-FABP are associated with corresponding changes in HOMA %S and putatively free testosterone. To test this possibility, the cohort was first dichotomized at the median of A-FABP. In a second step, we selected 20 pairs of women from the low and high A-FABP group matched for BMI (rounded to the whole number). As a result of the matching procedure, the women in the two groups had the same BMI (ln BMI 3.20 ± 0.09 kg/m^2 vs 3.22 ± 0.09 kg/m^2, $P=0.163$), and were of comparable age ($P=0.08$), but the A-FABP levels were significantly different (ln A-FABP 2.85 ± 0.08 ng/ml vs 3.75 ± 0.13 ng/ml, $P<0.001$; Fig. 1). Despite this highly significant difference between the two groups in circulating A-FABP, no differences in HOMA %S (ln HOMA %S 4.10 ± 0.12% vs 3.94 ± 0.14%, $P=0.3$) or in free testosterone (ln free testosterone 3.9 ± 0.20 pmol/l vs 3.76 ± 0.18 pmol/l, $P=0.6$) could be observed Fig. 1).

During lifestyle intervention insulin sensitivity significantly changed (ln HOMA %S 3.79 ± 0.15–3.36 ± 0.12, $P=0.009$) after 16 weeks in 17 obese PCOS women. The A-FABP levels, (ln A-FABP 3.84 ± 0.12–3.77 ± 0.15 ng/ml, $P=0.34$) as well as the BMI (ln BMI 3.66 ± 0.04–3.65 ± 0.04 kg/m^2, $P=0.11$) however, remained unchanged.

Taken together, our data do not support a major impact of circulating A-FABP on HOMA %S, hsCRP or free testosterone. Therefore, it appears unlikely that A-FABP is the molecular link between adiposity, IR, subclinical inflammation, and hyperandrogenemia in PCOS women.

Discussion

Adiposity, IR, and hyperandrogenism are key features in many women with PCOS (3, 4, 7). There is strong evidence that adiposity-related IR and consecutive hyperinsulinemia facilitate the development and maintenance of the syndrome. Therefore, it appears that at least in a subgroup of PCOS patients, the syndrome can be regarded as an obesity-related disorder. The molecular mechanisms, however, linking adiposity with obesity-related disorders are incompletely understood.

Animal studies suggested that A-FABP, which is expressed in the cytosol of adipocytes and macrophages, is a central regulator of systemic insulin sensitivity, lipid metabolism, and inflammatory responses associated with atherosclerosis (16–21). Recent data from humans demonstrate that A-FABP is released into the circulation and that the concentrations are significantly higher than those reported for most other adipocytokines (23, 24). Thus, a hypothesis was generated that circulating A-FABP may contribute to disorders associated with obesity in humans.

In non-diabetic PCOS women, plasma A-FABP was strongly associated with the degree of adiposity as measured by BMI. Further analysis using body composition data obtained by DEXA demonstrated a significant association of A-FABP with both DEXA-total fat mass and DEXA-lean body mass. Since the correlation of A-FABP with DEXA-total fat mass was significantly higher than the one with DEXA-lean body mass, and since A-FABP expression has only been reported from adipocytes and macrophages, the results are consistent with the concept that A-FABP is released from adipose tissue and is a biomarker of the degree of adiposity (23, 24). The most likely explanation for the association of A-FABP with DEXA-lean body mass seems to be the fact that in obese PCOS women lean body mass is higher than in non-obese patients (BMI versus DEXA-lean body mass $R=0.638$, $P<0.001$).

In our non-diabetic PCOS women, insulin sensitivity ranged from very sensitive (232.0 %S) to highly resistant (13.6 %S) as judged by HOMA %S (mean 78.3 ± 4.6 %S). This was confirmed by CIGMA %S which provides additional information about IR in the stimulated state (mean 54.0 ± 3.6 %S, range 1.0–203.0 %S). A-FABP correlated with both parameters of insulin sensitivity even after adjustment for age, smoking habits, and the state of glucose metabolism (NGT versus IGT). The correlations, however, were no longer significant after the inclusion of BMI into the regression models, which is in line with findings from Chinese individuals of both sexes (23). Furthermore, in BMI-matched women, differences in circulating A-FABP, although highly significant, were not paralleled by differences in IR. In addition, A-FABP levels did not parallel the changes in insulin sensitivity observed in women undergoing a lifestyle intervention program over 4 months. Thus, our data do not support the

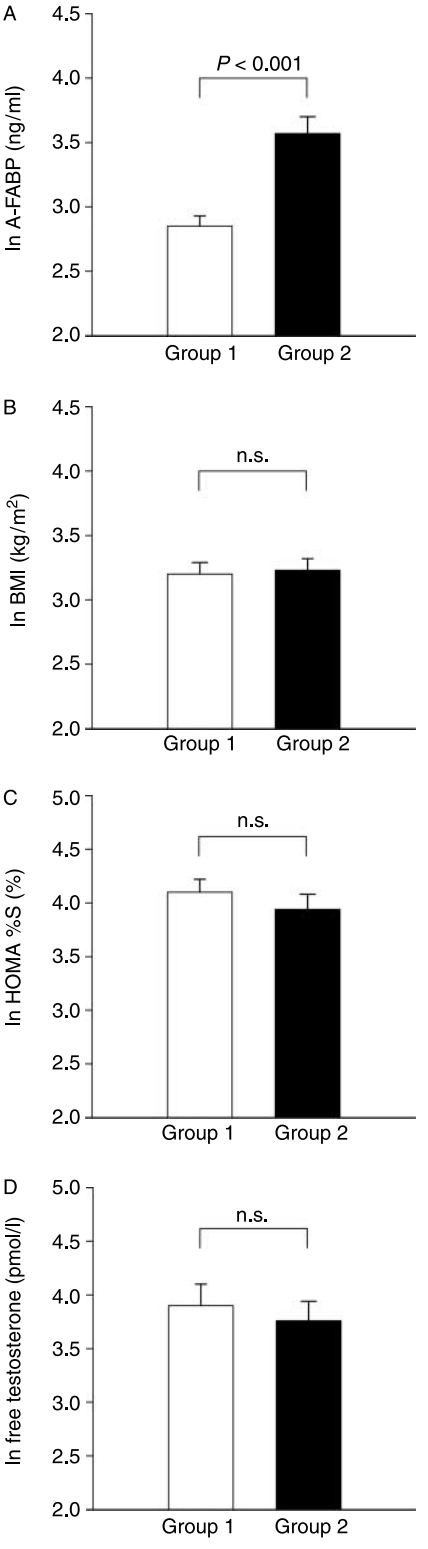

Figure 1 Comparison of HOMA %S and free testosterone between women in the low A-FABP group (group 1) and women in the high A-FABP group (group 2) matched by BMI (20 pairs). n.s., not significant.

hypothesis that circulating A-FABP is a major factor that links obesity with IR in PCOS women.

In PCOS, markers of obesity are associated with hyperandrogenemia (3, 4, 7, 40, 41). Adjusted for age, smoking habits, and the state of glucose metabolism the relation of A-FABP with free testosterone was no longer significant. Furthermore, similar to IR, no evidence for an independent impact of A-FABP on free testosterone was found in BMI-matched women with different A-FABP levels. Taken together, it is therefore unlikely that A-FABP is a significant link between adiposity and hyperandrogenism in PCOS women.

A-FABP, which is also expressed in macrophages, has been implicated in inflammatory responses associated with obesity and metabolic disorders (18). In our cohort, however, no association of circulating A-FABP with hsCRP could be delineated after the adjustment for potential confounders such as age, smoking, or glucose metabolism.

In summary, our data indicate that circulating A-FABP correlates well with BMI and total body fat mass in PCOS women, thereby confirming previous results that demonstrate a close relationship between markers of obesity and A-FABP concentrations in both sexes (23, 24). No evidence, however, could be found that circulating A-FABP provides a molecular mechanism linking adiposity to IR, subclinical inflammation, or hyperandrogenemia. Despite its relatively small size, our PCOS cohort appeared to be well suited for the investigations performed, since the cohort was rather homogenous and covered a wide range of insulin sensitivity and associated metabolic alterations. However, our data do not preclude the possibility that circulating A-FABP is an important mediator in adiposity-dependent processes other than the ones examined here. Furthermore, the findings also do not rule out a major role for intracellular A-FABP in the metabolic regulation of insulin sensitivity, dyslipidemia, and inflammation, as suggested by genetic studies in humans (22).

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