Decreased lipin 1β expression in visceral adipose tissue is associated with insulin resistance in polycystic ovary syndrome

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Abstract

Objective: In polycystic ovary syndrome (PCOS), insulin resistance (IR) appears with high prevalence and represents the major cause of cardiometabolic complications. Lipin 1β regulates lipid metabolism and augments insulin sensitivity. The impact of lipin 1β expression in visceral and subcutaneous adipose tissue of PCOS patients on IR was studied for the first time.

Methods: Eighty-five PCOS patients and 44 controls were enrolled for subcutaneous tissue biopsy, of whom 25 patients and 30 controls also underwent visceral adipose tissue biopsy. Gene expression of lipin 1β was measured, together with that of peroxisome proliferator-activated receptor γ, lipoprotein lipase, hormone-sensitive lipase, adiponectin and glucose transporter 4 in subcutaneous and visceral adipose tissue. Markers of obesity, IR and PCOS were also measured.

Results: In PCOS patients, lipin 1β expression in both adipose depots was lower than in controls: 0.76 (0.67–0.84) vs 1.16 (0.90–1.43) for visceral and 0.91 (0.73–1.10) vs 1.30 (1.03–1.57) for s.c. depot (both P < 10^-4). The difference remained significant after adjustment for body mass index (BMI) and also when comparing only lean patients with lean controls. In PCOS patients, visceral adipose lipin 1β expression correlated negatively with homeostasis model assessment–IR (r = -0.474, P = 0.017), BMI (r = -0.511, P = 0.009) and waist circumference (r = -0.473, P = 0.017), waist circumference remaining significant (P = 0.027) in multiple regression. Subcutaneous lipin 1β expression in PCOS correlated negatively with BMI, waist circumference and plasma triglycerides, and positively with high density lipoprotein-cholesterol. Subcutaneous, but not visceral lipin 1β expression, correlated positively with the studied genes.

Conclusions: Lipin 1β appears to be involved in the pathogenesis of IR in PCOS.

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Introduction

Insulin resistance (IR) is found in more than 50% of patients with polycystic ovary syndrome (PCOS), thus being an important component of PCOS and accounting for the increased risk of type 2 diabetes and cardiovascular disease in these patients (1, 2). Additionally, central obesity is a common feature in PCOS that further increases IR and is associated with unfavourable metabolic profile (3).

Lipin 1 is a newly discovered protein that is involved in lipid metabolism. It acts as a Mg2+-dependent phosphatidate phosphatase type 1 that hydrolyzes phosphatidate to diacylglycerol, thus playing a key role in the synthesis of triglycerides (TG) and phospholipids (4). In addition, lipin 1 can act as a transcription coactivator by directly interacting with peroxisome proliferator-activated receptor α (PPARA) and PPARG, coactivator 1 α (PPARGC1A) in the liver, increasing fatty acid oxidation (5). By favourably modifying lipid metabolism, lipin 1 augments insulin sensitivity. A combination of mutations in the lipin 1 gene causes lipin deficiency with IR and fatty liver dystrophy in mice (6).

Expression studies revealed two distinct products of the lipin 1 gene. Lipin 1α is the predominant isoform in preadipocytes. It stimulates adipocyte differentiation by inducing the genes for PPARA and the CCAAT/ enhancer-binding protein α (CEBPA) (7). Lipin 1β is the predominant isoform in mature adipocytes, where it increases the expression of genes involved in TG and free fatty acid synthesis and consequent lipid accumulation (7).

In humans, lipin 1β expression in s.c. adipose tissue (8–12) and in the liver (11) has been shown to be inversely correlated with obesity and IR. Lipin 1β expression in s.c. adipose tissue can be reactivated by weight loss (10, 11) and thiazolidinedione treatment (9). This led us to explore lipin 1β mRNA expression in adipose tissue of PCOS patients.
None of the studies exploring human lipin 1 has analyzed correlations of visceral adipose tissue lipin 1 expression with markers of IR. Although visceral adiposity is the main cause of IR, type 2 diabetes and cardiovascular complications (13–15).

Given the strong negative association of lipin 1b with IR, we have explored whether lipin 1β expression in visceral adipose tissue is decreased in PCOS patients, thus contributing to IR. The correlation of lipin 1β expression in visceral and s.c. adipose tissue with markers of IR and obesity in PCOS was assessed. Additionally, lipin 1β relations with the lipid metabolism genes PPARG, lipoprotein lipase (LPL), hormone-sensitive lipase (LIPE), adiponectin and glucose transporter 4 (GLUT4) genes expression in both adipose tissues were studied.

**Methods of biochemical markers and hormones determination**

Glucose levels were determined using a standard glucose oxidase method (Roche Hitachi 917, Roche). A chemiluminescent immunoassay was used to measure plasma insulin (Liaison Insulin, Diasorin, Salluggia, Italy). Androstenedione and DHEAS were measured by a specific double antibody RIA using 125 I-labelled hormones (Diagnostic Systems Laboratories, Webster, TX, USA). Total and free testosterone levels were determined by RIA (DiaSorin and DPC, Los Angeles, CA, USA). HsCRP and SHBG were assessed by chemiluminescent immunoassay (Immulite, DPC). Total cholesterol and TG concentrations were measured by enzymatic–colorimetric methods; high density lipoprotein (HDL)-cholesterol was measured by a direct method (Roche Hitachi 917, Roche). Low density lipoprotein (LDL)-cholesterol was determined by the Friedewald formula. Intraassay variations ranged from 1.6 to 6.3%, and interassay variations ranged from 5.8 to 9.6% for the applied methods.

The homeostasis model assessment-IR (HOMA-IR) score was used to determine IR using the formula: fasting serum insulin (mU/l) x fasting plasma glucose (mmol/l)/22.5 (18). HOMA-IR score value of 2.18 was considered a cut-off point for IR (19).

**RNA analysis**

Total RNA from adipose tissue was isolated using RNeasy Lipid Tissue Mini kit (Qiagen) according to the manufacturer’s instructions. The quantity and the quality of isolated RNA were determined by a Nanodrop ND-1000 (Thermo Fisher Scientific) and an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) respectively. Total RNA was transcribed to cDNA using TaqMan Reverse Transcript reagents (Applied Biosystems, Foster City, CA, USA). Real time PCR was performed on ABI PRISM 7000 Sequence Detection System (Applied Biosystems) using primers and probes of inventoried or predesigned assays for PPARG (mRNA isoform 2, Hs01115510_m1), LPL (Hs00173425_m1), LIPE (Hs00943410_m1), adiponectin (ADIPOQ, Hs00605917_m1) and glucose transporter 4 (SLC2A4, Hs00168966_m1). Primers and probes for lipin 1β were synthesized on request (primers 5’T-AGGCTCATACCTAATTCGGGATA, 5’R-GGCAACCTCCTTTTGCAATCTACCA and a probe, 5’-AccACCTCC-CAGTAGCC, Applied Biosystems). Cyclophilin A and phosphoglycerate kinase 1 (TaqMan Endogenous
Controls, Applied Biosystems) were used as housekeeping genes. Each measurement was run in duplicate in a 20 μl reaction mixture with 10 ng total RNA converted to cDNA. Quantification was done using a calibration curve. The expression of target genes was reported relative to a normalization factor based on housekeeping gene expression (20).

In the 44 control subjects only lipin 1β mRNA expression in obtained adipose tissues was assessed, to be compared with that in PCOS patients. Correlations of lipin 1β expression with biochemical and anthropometric markers were not studied in the control group.

**Statistical procedures**

The data for lipin 1β expression and biochemical parameters were not normally distributed; therefore nonparametric statistical tests were used. The groups were compared using Mann–Whitney test, while s.c. and visceral gene expressions were compared by Wilcoxon signed-ranks test. Adjustments for BMI and age in the comparison of lipin 1β expression between patients and controls were performed using logistic regression. To test associations between continuous variables Spearman’s correlation were calculated. The stepwise multiple regression analysis with log transformed variables was done to evaluate which clinical parameter had stronger correlation with lipin 1β expression. Statistical analyses were performed using SPSS software version 15.0 (Chicago, IL, USA). Data are expressed as medians (lower–upper quartile). A P value ≤ 0.05 was considered statistically significant.

**Results**

**Lipin 1β expression in adipose tissue of PCOS patients and controls**

The expression of lipin 1β in s.c. adipose tissue was measured in 129 subjects. Clinical and biochemical characteristics of controls (n = 44) and PCOS patients (n = 85) are presented in Table 1. Lipin 1β expression in visceral adipose tissue was analyzed in 55 subjects: 30 controls and 25 PCOS patients.

PCOS patients exhibited lower s.c. (P < 10⁻⁶) and visceral (P < 10⁻⁴) adipose lipin 1β expression than controls (Fig. 1A). After adjustment for BMI and age in logistic regression, s.c. lipin 1β expression remained a significant predictor of PCOS or control status (P = 0.001, odds ratio (OR; PCOS versus control) = 0.110, 95% CI: 0.031–0.388), i.e. when s.c. lipin 1 expression increases by 1 unit, the odds of PCOS to odds of healthy state decreases by a factor of 0.110. After adjustment for BMI and age, visceral lipin 1β expression also remained associated with the disease status (P = 0.021, OR (PCOS versus control) = 0.027, 95% CI: 0.001–0.584).

Testing only lean controls compared with lean PCOS patients (BMI < 25 kg/m²) for lipin 1β expression, PCOS patients again showed lower s.c. lipin 1β expression (patients: 1.04 (0.74–1.21), n = 33; controls 1.31 (1.03–1.57), n = 39; P = 0.001) and visceral lipin 1β expression (patients 0.81 (0.79–1.14), n = 11: controls: 1.19 (0.91–1.44), n = 27; P = 0.006). Lean patients and controls did not differ in BMI (P = 0.220).

Comparing the levels of lipin 1β expression in both types of adipose tissue, lipin 1β expression was higher in s.c. than in visceral compartment in PCOS patients.
However, no significant difference was found between both adipose compartments in controls (data not shown).

**Correlation of lipin 1β expression with anthropometric and metabolic markers in PCOS patients**

Lipin 1β expression in visceral adipose tissue of PCOS patients correlated negatively with BMI and waist circumference and positively with plasma HDL-cholesterol (Table 2). Similar relations were observed with lipin 1β expression in s.c. adipose tissue where a negative correlation with plasma TG also emerged (Table 2). However, only lipin 1β expression in visceral adipose tissue correlated negatively with fasting plasma glucose, insulin and HOMA-IR and, additionally, with hsCRP. A trend for positive correlation with SHBG was evident (Table 2). In stepwise multiple regression, with lipin 1β expression in visceral adipose tissue as a dependent variable, waist circumference was the only significant predictor with standardized $\beta = -0.442$ and $P = 0.027$. Lipin 1β expression in s.c. adipose tissue showed significant association in multiple regression only with HDL-cholesterol ($\beta = 0.355$, $P = 0.001$).

**Correlation of lipin 1β expression with expression of other genes in PCOS patients**

The correlation of lipin 1β expression with the expression of genes responsible respectively for lipid tissue differentiation (PPARG), lipid uptake (LPL) and lipolysis (LIPE), of a gene encoding insulin sensitivity enhancer adiponectin and a gene implicated in cellular glucose uptake (SLC2A4) was analyzed. The expression of lipin 1β in s.c. adipose tissue correlated positively with PPARG, LPL, LIPE, adiponectin and SLC2A4 gene expression (Table 3). In visceral adipose tissue, lipin 1β expression showed no correlation with the expression of these genes (data not shown).

**Discussion**

The present study is the first to assess the role of lipin 1 in PCOS and to examine lipin 1 expression in visceral and s.c. adipose tissue with regard to metabolic and anthropometric parameters. In both adipose tissues of our PCOS patients, the gene expression of lipin 1β was decreased. Lipin 1β expression showed a strong negative correlation with markers of obesity, and also with markers of IR. These findings indicate that lipin 1β could be involved in the pathogenesis of IR in women with PCOS.

Lipin 1 is a recently discovered protein regulating lipid metabolism by playing a key role in the synthesis of TG and phospholipids (4). In addition, lipin 1 can increase fatty acid oxidation in the liver (5). By favourably modifying lipid metabolism, lipin 1 augments insulin sensitivity. In our study of PCOS patients, the metabolic activity of visceral and s.c. adipose tissue was examined through lipin 1β expression.

Our PCOS patients had lower lipin 1β expression in s.c. and visceral adipose tissue compared with controls. Lipin 1β expression in both types of adipose tissue correlated negatively with markers of obesity, BMI and waist circumference, as expected. However, only lipin 1β expression in visceral adipose tissue showed a negative correlation with the markers of IR: HOMA-IR, plasma insulin, glucose and hsCRP. By multivariate analysis, the negative correlation of visceral lipin 1β expression with waist circumference remained significant, indicating that progressive visceral adiposity.
exerts its deleterious metabolic effects, without being opposed by lipin 1 activity. Namely, when the normal size of an adipocyte is exceeded, lipin 1 expression may be progressively attenuated, resulting in the reverse relationship between adiposity and lipin 1 expression, as proposed by Donkor et al. (12). The lack of lipin 1 activity to stimulate TG synthesis and lipid accumulation shifts the lipids towards liver and muscle causing IR in these tissues. In the previous study (12), some evidence was presented for lipin 1 inducing free fatty acid catabolism in adipose tissue, which could also account for the negative relationship of lipin 1 expression with obesity and IR found in our study.

After adjusting for BMI and comparing only our lean PCOS patients with our lean controls, lipin 1 β expression was still significantly lower in the patients, suggesting that lack of lipin 1 may play a role in the pathogenesis of IR in PCOS.

In our PCOS patients, lipin 1 β expression correlated negatively with plasma TG and positively with HDL-cholesterol. This supports the notion of the beneficial effects of lipin 1 β on atherogenic dyslipidaemia manifested by low HDL-cholesterol, small dense LDL particles and elevated levels of TG, being one of the major cardiovascular risk factors in the states of IR including PCOS (21). Other research groups, exploring only the s.c. fat tissue, found negative correlations of lipin 1 expression with TG, BMI, waist circumference and markers of IR in healthy (9, 12), morbidly obese subjects (10, 11), and in dyslipidaemic patients (8). Some of these correlations are in concordance with our findings.

Table 2: Spearman’s correlation coefficients and significance levels of correlation between lipin 1β expression in visceral/s.c. adipose tissue and metabolic phenotype in polycystic ovary syndrome (PCOS).

<table>
<thead>
<tr>
<th>Lipin 1β expression in visceral adipose tissue (n=25)</th>
<th>Lipin 1β expression in s.c. adipose tissue (n=85)</th>
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<tbody>
<tr>
<td><strong>r</strong></td>
<td><strong>P</strong></td>
</tr>
<tr>
<td>BMI</td>
<td>-0.511</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>-0.473</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>-0.322</td>
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<tr>
<td>Diastolic BP</td>
<td>-0.255</td>
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<tr>
<td>Fasting glucose</td>
<td>-0.413</td>
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<tr>
<td>Fasting insulin</td>
<td>-0.428</td>
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<tr>
<td>HOMA-IR</td>
<td>-0.474</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.159</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.544</td>
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<tr>
<td>LDL cholesterol</td>
<td>0.065</td>
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<tr>
<td>TG</td>
<td>-0.336</td>
</tr>
<tr>
<td>HsCRP</td>
<td>-0.449</td>
</tr>
<tr>
<td>Total testosterone</td>
<td>-0.219</td>
</tr>
<tr>
<td>Free testosterone</td>
<td>-0.162</td>
</tr>
<tr>
<td>SHBG</td>
<td>0.392</td>
</tr>
<tr>
<td>DHEAS</td>
<td>0.182</td>
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<tr>
<td>Androstenedione</td>
<td>0.139</td>
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</tbody>
</table>

Significances at 0.05 level are shown in bold. TG, triglycerides; hsCRP, high sensitivity C-reactive protein; SHBG, sex hormone-binding globulin; DHEAS, DHEA-sulphate.

In our PCOS patients, positive correlations of lipin 1β expression with lipid metabolism genes PPARG, LPL, LIPE and the gene for insulin sensitivity augmenting protein adiponectin were found in s.c. adipose tissue. This could be partly explained by the evidence that PPARG activity induces the genes for lipin 1 (lipin 1 mRNA (9), LPL (23), LIPE (24) and adiponectin in s.c. adipose tissue (25). On the other hand, in the state of lipin 1 deficiency in the fatty liver dystrophy mice, diminished LPL activity in adipose tissue (26) and decreased plasma adiponectin concentration were observed along with severe IR (27).

Table 3: Spearman’s correlation coefficients and significance levels of correlation between lipin 1β expression and the expression of lipid and glucose metabolism genes in s.c. adipose tissue.

<table>
<thead>
<tr>
<th>Gene</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPARG</td>
<td>0.311</td>
<td>0.015</td>
</tr>
<tr>
<td>LPL</td>
<td>0.402</td>
<td>&lt;10^−4</td>
</tr>
<tr>
<td>LIPE</td>
<td>0.486</td>
<td>&lt;10^−5</td>
</tr>
<tr>
<td>ADIPOQ</td>
<td>0.476</td>
<td>0.016</td>
</tr>
<tr>
<td>SLC2A4</td>
<td>0.608</td>
<td>&lt;10^−5</td>
</tr>
</tbody>
</table>

PPARG, peroxisome proliferator-activated receptor γ; LPL, lipoprotein lipase; LIPE, hormone-sensitive lipase; ADIPOQ, adiponectin; SLC2A4, glucose transporter 4.
Lipin 1β expression in s.c. adipose tissue of our PCOS patients also correlated positively with the gene expression of glucose transporter 4 (SLC2A4) – the last step in the insulin signalling cascade. SLC2A4 expression is a marker of tissue insulin sensitivity and was shown to correlate negatively with HOMA-IR in PCOS patients (28). The mechanism of this correlation remains to be elucidated. However, the absence of correlation between lipin 1β and the remaining genes expression in visceral adipose tissue observed in our study could be attributed to different regulatory mechanisms in this adipose tissue compartment or could be due to the smaller number of PCOS patients in whom visceral adipose tissue was studied.

A positive association of lipin 1β expression in visceral adipose tissue with plasma SHBG was also found in our PCOS patients. SHBG is usually low in states of IR since compensatory hyperinsulinemia suppresses liver SHBG synthesis (29).

In conclusion, our study revealed several indices pointing towards lipin 1β involvement in the pathogenesis of IR in PCOS: negative correlations of lipin 1β expression in visceral adipose tissue with serum glucose, insulin and HOMA-IR; and positive correlations with SLC2A4 expression in visceral adipose tissue observed in our study. Lipin 1β with its favourable effects on lipid metabolism protects from the development of IR. Low lipin 1β expression as found in PCOS patients thus promotes the opposite.

Declaration of interest
We declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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