Pioglitazone (7.5 mg/day) added to flutamide–metformin in women with androgen excess: additional increments of visfatin and high molecular weight adiponectin

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Summary

Background and aim Low-dose pioglitazone (Pio), flutamide (Flu), metformin (Met) plus an oestro-progestagen is a novel polytherapy lowering total and visceral adiposity, and reducing carotid intima media thickness (IMT) in hyperinsulinaemic women with androgen excess, without changing their body mass index (BMI). In a search for mediators of PioFluMet’s actions, we measured serum levels of visfatin and high molecular weight (HMW) adiponectin.

Design and patients In a double-blind study, we enrolled 38 young women with hyperinsulinaemic androgen excess [mean BMI: 23.7 kg/m²], all of whom started on Flu (62.5 mg/day), Met (850 mg/day) and a transdermal oestro-progestagen, each for 21/28 days over 1 year. Patients were randomly assigned to receive, in addition, placebo (n = 19) or Pio (7.5 mg/day; n = 19) on the same 21/28 days.

Measurements Serum concentrations of visfatin and HMW adiponectin, visceral fat by magnetic resonance imaging, carotid IMT by ultrasound, all carried out during study start and after 1 year.

Results PioFluMet raised visfatin by a mean 84% and HMW adiponectin by 157% (P < 0.001), and reduced visceral fat and IMT by a mean 22% and 31% (both P < 0.001). Low-dose Pio accounted for about half of the PioFluMet effects on IMT, visfatin and HMW adiponectin.

Conclusion In hyperinsulinaemic women with androgen excess, low-dose polytherapy with PioFluMet evoked striking rises in both circulating visfatin and HMW adiponectin, while lowering IMT and reducing visceral adiposity within 1 year.

Introduction

Hyperinsulinaemic androgen excess in young women can be treated with a combination of insulin sensitization and androgen-receptor blockade and, if needed, lifestyle changes and contraceptive measures.1–3 Additive insulin sensitization with low-dose pioglitazone (Pio) (7.5 mg/day), is accompanied by additional improvements in the endocrine-metabolic state, in low-grade inflammation and in markers of cardiovascular health,4 but evidence beyond 6 months is lacking, and the mediating mechanisms are unknown.

In a search for mediators of PioFluMet’s actions, we measured the serum levels of two newly identified adipocytokines, visfatin and high molecular weight (HMW) adiponectin, that have been linked to insulin resistance, inflammation and cardiovascular risk in several disorders, including hyperinsulinaemic androgen excess. Adiponectin has anti-atherogenic and insulino-mimetic properties; the secretion of adiponectin’s active HMW isoform by adipocytes in vitro is up-regulated by Pio and down-regulated by testosterone.5,6 Visfatin seems to be more expressed in visceral than subcutaneous fat, to promote adipogenesis, and to exert insulin-mimetic effects, but its role in human metabolism is controversial and poorly defined;7–9 for example, administration of Pio and other thiazolidinediones to both healthy and diabetic individuals has been found to exert either no effects on visfatin or to increase visfatin release.5,9

Subjects, materials and methods

Patients and ethics

The patients were 38 young women with hyperinsulinaemic androgen excess (mean ± SEM; 19.6 ± 0.3 years, range 18–24 years;
Table 1. Results in hyperinsulinaemic women with androgen excess, who received treatment with low-dose FluMet (Flu, 62.5 mg/day; Met, 850 mg/day) and an oestro-progestagen, and who were randomized to receive in addition either placebo (21/28 days; 19 patients) or low-dose pioglitazone (Pio, 7.5 mg/day; 21/28 days; n = 19) for 1 year

<table>
<thead>
<tr>
<th></th>
<th>All at start</th>
<th>FluMet + oestro-progestagen + placebo</th>
<th>FluMet + oestro-progestagen + Pio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>at start‡</td>
<td>1 year 0–1 year</td>
<td>at start* 1 year 0–1 year</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>19.6 ± 0.3</td>
<td>19.2 ± 0.3 20.2 ± 0.3 1.0 ± 0.0</td>
<td>19.9 ± 0.5 20.9 ± 0.5 1.0 ± 0.0</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>23.7 ± 0.5</td>
<td>23.1 ± 0.6 23.0 ± 0.7 –0.1 ± 0.4</td>
<td>24.3 ± 0.6 24.5 ± 0.7 0.2 ± 0.2</td>
</tr>
<tr>
<td><strong>HOMA-IR</strong></td>
<td>2.8 ± 0.2</td>
<td>2.5 ± 0.3 2.1 ± 0.2 –0.4 ± 0.3</td>
<td>3.1 ± 0.2 2.3 ± 0.2† –0.7 ± 0.3</td>
</tr>
<tr>
<td><strong>LDL : HDL ratio</strong></td>
<td>2.1 ± 0.1</td>
<td>2.1 ± 0.1 1.8 ± 0.1 –0.2 ± 0.1</td>
<td>2.2 ± 0.1 1.6 ± 0.1§ –0.6 ± 0.1§</td>
</tr>
<tr>
<td><strong>Testosterone (nmol/l)</strong></td>
<td>2.9 ± 0.1</td>
<td>2.8 ± 0.2 1.7 ± 0.2§ –1.1 ± 0.2</td>
<td>2.9 ± 0.2 1.6 ± 0.1§ –1.3 ± 0.2</td>
</tr>
<tr>
<td><strong>SHBG (nmol/l)</strong></td>
<td>36 ± 2</td>
<td>37 ± 3 169 ± 8§ 132 ± 7</td>
<td>35 ± 3 172 ± 5 137 ± 5</td>
</tr>
<tr>
<td><strong>Androstenedione (nmol/l)</strong></td>
<td>16.2 ± 0.8</td>
<td>15.9 ± 1.1 10.2 ± 0.8§ –5.7 ± 1.0</td>
<td>16.4 ± 1.3 9.3 ± 0.7§ –7.1 ± 1.0</td>
</tr>
<tr>
<td><strong>DHEAS (μmol/l)</strong></td>
<td>7.2 ± 0.5</td>
<td>7.2 ± 0.6 4.6 ± 0.3§ –2.6 ± 0.5</td>
<td>7.1 ± 0.8 5.3 ± 0.7 –1.8 ± 0.5</td>
</tr>
<tr>
<td><strong>ALT (U/l)</strong></td>
<td>15.1 ± 0.8</td>
<td>14.3 ± 1.1 14.2 ± 1.0 –0.1 ± 0.4</td>
<td>15.8 ± 1.3 14.2 ± 1.4 –1.6 ± 1.8</td>
</tr>
<tr>
<td><strong>Haematocrit (%)</strong></td>
<td>40.1 ± 0.4</td>
<td>40.0 ± 0.4 38.5 ± 0.5§ –1.5 ± 0.4</td>
<td>40.3 ± 0.6 38.6 ± 0.8† –1.7 ± 0.7</td>
</tr>
<tr>
<td><strong>Neutro : Lympho ratio</strong></td>
<td>3.7 ± 0.3</td>
<td>3.8 ± 0.2 2.9 ± 0.4§ –0.9 ± 0.4</td>
<td>3.7 ± 0.5 2.2 ± 0.3 –1.5 ± 0.4</td>
</tr>
<tr>
<td><strong>HMW-adiponectin (mg/l)</strong></td>
<td>7 ± 1</td>
<td>7 ± 1 12 ± 2‡ 5 ± 2</td>
<td>7 ± 1 18 ± 2§ 11 ± 2**</td>
</tr>
<tr>
<td><strong>Visfatin (μg/l)</strong></td>
<td>46 ± 3</td>
<td>48.5 ± 5 62.4 ± 14 14 ± 6</td>
<td>43 ± 3 79 ± 8§ 36 ± 10¶</td>
</tr>
<tr>
<td><strong>IMT (mm)</strong></td>
<td>0.47 ± 0.01</td>
<td>0.46 ± 0.01 0.40 ± 0.00§ –0.06 ± 0.01</td>
<td>0.48 ± 0.02 0.33 ± 0.01§ –0.15 ± 0.02‡</td>
</tr>
<tr>
<td><strong>L3 Visc fat (cm²)</strong></td>
<td>53 ± 3</td>
<td>51 ± 3 42 ± 3‡ –9 ± 3</td>
<td>54 ± 5 42 ± 4§ –12 ± 2</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

BMI, body mass index; HOMA-IR, homeostasis model assessment-insulin resistance; SHBG, sex hormone-binding globulin; DHEAS, dehydroepiandrosteronesulphate; ALT, alanine aminotransferase; Neutro, neutrophil count; Lympho, lymphocyte count; CRP, C-reactive protein; HMW, high molecular weight; IMT, intima media thickness in left carotid artery; L3, lumbar vertebra 3 level; Visc, visceral.

Indicative values from asymptomatic young women: testosterone, 1.1 ± 0.1 nmol/l (5–12 years postmenarche; Table 1).

*Absence of significant differences between randomized subgroups at baseline.

‡P ≤ 0.05, §P ≤ 0.01, ¶P ≤ 0.001 vs. baseline. **P ≤ 0.05 for 0–1 year change (Δ) vs. placebo.

Prior to study start, none of the patients was receiving a contraceptive or another medication known to affect gonadal or adrenal function, or carbohydrate or lipid metabolism, for at least 9 months.

This study was registered as ISRCTN12871246 and conducted in Barcelona, without support from the industry, after approved by the Institutional Review Board of Sant Joan University Hospital, and after informed consent by each of the patients.

**Study design and treatment**

In this double-blind study, women started on metformin (Met) (850 mg/day) and flutamide (Flu) (62.5 mg) once daily (21/28 days; dinner time), and a transdermal contraceptive with ethinylestradiol 600 μg plus norelgestromin 6 mg, via a weekly patch (Evra, Janssen-Cilag, 21/28 days) for 1 year. After stratification for BMI, patients were randomly assigned to receive, in addition, placebo (21/28 days; n = 19) or Pio (7.5 mg, 21/28 days; n = 19), at breakfast time, for 1 year. FluMet and placebo/Pio were discontinued during the week-off contraception.

The randomization sequence was unknown to clinically involved investigators. Pio and placebo were packaged in similar tablets; renewals were scheduled 3-monthly. All patients and investigators, except for the study statistician (AL-B), have so far been (and still are) blinded to placebo/Pio allocation.

Clinical and endocrine-metabolic variables, carotid intima media thickness (IMT) and visceral fat were all assessed during the study start [in follicular phase (days 3–7) or after 2 months of amenorrhoea],
used a high-resolution apparatus with colour and power Doppler by the same investigator (GE, blinded to treatment allocation) who and the top of the vessels above the vertebrae as guides for the spline fit. VAT area was calculated by subtracting the organ areas from the intraperitoneal area.

**Clinical and endocrine assessment**

Height (by stadiometer), weight, BMI, WHR and hirsutism score were assessed by the same investigator (LI, blinded to treatment allocation). Fasting blood glucose, neutrophil and lymphocyte count, serum insulin, LDL- and HDL-cholesterol, SHBG, testosterone, dehydroepiandrosterone sulphate (DHEAS), C-reactive protein (CRP), visfatin and HMW adiponectin were measured with alanine- and aspartate aminotransferase (ALT, AST), γ glutamyl transpeptidase (GGT), besides a screening of renal function. Fasting insulin resistance was estimated from fasting insulin and glucose levels using the homeostasis model assessment (HOMA).10

**IMT and visceral fat**

As described,7 ultrasound scans of the carotid arteries were obtained by the same investigator (GE, blinded to treatment allocation) who used a high-resolution apparatus with colour and power Doppler capabilities (Acuson Sequoia 512 SHA, Los Alamitos, CA) and a high-frequency 10 MHz linear probe. Visceral adipose tissue (VAT) was assessed at L3 level, by magnetic resonance, using a multislice 1-5 Tesla device (Signa LX Echo Speed Plus Excite, General Electric, Milwaukee, WI). VAT area was measured by fitting a spline curve to points on the border of the visceral regions, selected by the same operator (blinded to treatment allocation). Nonfat regions within the visceral region were also outlined with a spline fit and subtracted from the total visceral region. The visceral fat region was subdivided into retroperitoneal and intraperitoneal areas using the ascending and descending colons, the psoas muscles on each side of the spine, and the top of the vessels above the vertebrae as guides for the spline operator (blinded to treatment allocation). Nonfat regions within points on the border of the visceral regions, selected by the same

**Assays and statistics**

Neutrophil and lymphocyte counts, serum glucose, insulin, testosterone, DHEAS, androstenedione, SHBG and CRP were measured as described;6 methods had intra- and interassay CVs between 4% and 8%. Visfatin was measured by an ELISA (EIA kit; Phoenix, Belmont, CA) with CVs < 6%; HMW adiponectin was measured by sandwich ELISA (Linco, St Charles, MO) with CVs < 9%. Statistical analyses were performed with spss 12.0 (SPSS Inc, Chicago, IL). For uniformity, results are expressed as mean ± SEM. Within-group changes over 1 year were tested by paired-samples t-test. Between-group differences in changes over 1 year were tested by two-way (time and group) repeated-measures ANOVA. Skewed data were log-transformed into normal distributions prior to comparisons. Significance level was set at P < 0.05.

**Results**

Results over 1 year are listed in Table 1. On average, low-dose polytherapy with Pio raised visfatin by 84% and HMW adiponectin by 157%, and reduced visceral fat by 22% and IMT by 31%. Without changing BMI, low-dose Pio accounted for about half of the effects on IMT, visfatin and HMW adiponectin (Fig. 1).

In the total study population (N = 38; data not shown), significant 1-year changes included decreases in WHR, hirsutism score, insulin, glucose, testosterone, LDL : HDL ratio, neutrophil : lymphocyte ratio and CRP, while hepatic markers as ALT, AST and GGT remained unchanged, indicating absence of hepatotoxicity. Clinical side effects were not detected; none of the patients dropped out of the study.

**Discussion**

Hyperinsulinaemic androgen excess is a long-term disorder that may have early origins, and that usually becomes symptomatic in adolescence. Low-dose polytherapy (plus lifestyle measures, if needed) is a long-term approach that primarily aims at preventing disease in later life, but is also an effective way of achieving short-term benefits in young women.1-4 Low-dose polytherapy with PioFluMet and an oestro-progestagen was here shown to be followed not only by a less hyperinsulinaemic, less hyperandrogenic, less pro-inflammatory, less atherogenic and viscerally less adipose state, but also by striking increments of circulating visfatin and HMW adiponectin.

The marked rise of HMW adiponectin in our patients may be conferred by the unprecedented combination of Pio and Flu. HMW adiponectin release by adipocytes is in vitro up-regulated by Pio and down-regulated by androgens.5,6 Androgen-receptor blockade with Flu was therefore expected to evoke an elevation of HMW adiponectin in hyperandrogenic women. Our clinical data now suggest that Pio-mediated up-regulation and Flu-mediated antidown-regulation of HMW adiponectin are additive, and they thus corroborate the rationale for a combined insulin-sensitizing plus anti-androgen therapy in conditions of hyperinsulinaemic
androgen excess. Circulating HMW adiponectin levels have been shown to also rise in obese women with polycystic ovary syndrome receiving short-term Pio (30 mg/day) in monotherapy.\textsuperscript{11}

Hyperandrogenic women have high serum visfatin concentrations and have a high fraction of visceral fat,\textsuperscript{12,13} as our patients had at start of treatment. It is unclear how PioFluMet treatment amplified the baseline hypervisfatinæmia. One possibility is that the PioFluMet-associated rise in circulating visfatin is an epiphenomenon of the reduction in visceral adiposity.\textsuperscript{14} The mechanisms underpinning the metabolic actions of thiazolidinediones are still controversial;\textsuperscript{29} at present, it is difficult to gauge to which extent the clinical benefits elicited by PioFluMet are mediated by hypervisfatinæmia.

In conclusion, in hyperinsulinaemic women with androgen excess, low-dose polytherapy with PioFluMet evoked striking rises in both circulating visfatin and HMW adiponectin, while lowering IMT and reducing visceral adiposity.

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References


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