

RAPID PUBLICATION

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

Lourdes Ibáñez*, Abel López-Bermejo†, Marta Díaz*, Goya Enríquez§, Carme Vallst and Francis de Zegher¶

*Endocrinology Unit and †Hormonal Laboratory, Hospital Sant Joan de Déu, University of Barcelona, Barcelona, Spain, ‡Diabetes, Endocrinology and Nutrition Unit, Dr Josep Trueta Hospital, Girona, Spain, §Department of Radiology, Hospital Materno-Infantil Vall d'Hebron, Autonomous University of Barcelona, Barcelona, Spain and ¶Department of Woman and Child, University of Leuven, 3000 Leuven, Belgium

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.

(Received 26 July 2007; returned for revision 11 September 2007; finally revised 17 September 2007; accepted 29 October 2007)

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 insulin-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.^{8,9}

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;

Correspondence: Lourdes Ibáñez, Endocrinology Unit, Hospital Sant Joan de Déu, University of Barcelona, Passeig de Sant Joan de Déu, 2, 08950 Esplugues, Barcelona, Spain. Tel.: +34 93 2804000, ext. 2261; Fax: +34 93 2033959; E-mail: libanez@hsjdbcn.org

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; $n = 19$) or low-dose pioglitazone (Pio, 7.5 mg/day; 21/28 days; $n = 19$) for 1 year

	All at start	FluMet + oestro-progestagen + placebo			FluMet + oestro-progestagen + Pio		
		at start ^a	1 year	0–1 year	at start ^a	1 year	0–1 year
Age (years)	19.6 ± 0.3	19.2 ± 0.3	20.2 ± 0.3	1.0 ± 0.0	19.9 ± 0.5	20.9 ± 0.5	1.0 ± 0.0
BMI (kg/m ²)	23.7 ± 0.5	23.1 ± 0.6	23.0 ± 0.7	−0.1 ± 0.4	24.3 ± 0.6	24.5 ± 0.7	0.2 ± 0.2
HOMA-IR	2.8 ± 0.2	2.5 ± 0.3	2.1 ± 0.2	−0.4 ± 0.3	3.1 ± 0.2	2.3 ± 0.2†	−0.7 ± 0.3
LDL : HDL ratio	2.1 ± 0.1	2.1 ± 0.1	1.8 ± 0.1	−0.2 ± 0.1	2.2 ± 0.1	1.6 ± 0.1\$	−0.6 ± 0.1¶
Testosterone (nmol/l)	2.9 ± 0.1	2.8 ± 0.2	1.7 ± 0.2\$	−1.1 ± 0.2	2.9 ± 0.2	1.6 ± 0.1\$	−1.3 ± 0.2
SHBG (nmol/l)	36 ± 2	37 ± 3	169 ± 8\$	132 ± 7	35 ± 3	172 ± 5\$	137 ± 5
Androstenedione (nmol/l)	16.2 ± 0.8	15.9 ± 1.1	10.2 ± 0.8\$	−5.7 ± 1.0	16.4 ± 1.3	9.3 ± 0.7\$	−7.1 ± 1.0
DHEAS (μmol/l)	7.2 ± 0.5	7.2 ± 0.6	4.6 ± 0.3\$	−2.6 ± 0.5	7.1 ± 0.8	5.3 ± 0.7\$	−1.8 ± 0.5
ALT (U/l)	15.1 ± 0.8	14.3 ± 1.1	14.2 ± 1.0	−0.1 ± 0.4	15.8 ± 1.3	14.2 ± 1.4	−1.6 ± 1.8
Haematocrit (%)	40.1 ± 0.4	40.0 ± 0.4	38.5 ± 0.5‡	−1.5 ± 0.4	40.3 ± 0.6	38.6 ± 0.8†	−1.7 ± 0.7
Neutro : Lympho ratio	1.9 ± 0.1	1.7 ± 0.1	1.3 ± 0.1†	−0.4 ± 0.2	2.1 ± 0.1	1.4 ± 0.1\$	−0.7 ± 0.2
CRP (mg/l)	3.7 ± 0.3	3.8 ± 0.2	2.9 ± 0.4†	−0.9 ± 0.4	3.7 ± 0.5	2.2 ± 0.3‡	−1.5 ± 0.4
HMW-adiponectin (mg/l)	7 ± 1	7 ± 1	12 ± 2‡	5 ± 2	7 ± 1	18 ± 2\$	11 ± 2**
Visfatin (μg/l)	46 ± 3	48 ± 5	62 ± 4†	14 ± 6	43 ± 3	79 ± 8‡	36 ± 10¶
IMT (mm)	0.47 ± 0.01	0.46 ± 0.01	0.40 ± 0.01\$	−0.06 ± 0.01	0.48 ± 0.02	0.33 ± 0.01\$	−0.15 ± 0.02**
L3 Visc fat (cm ²)	53 ± 3	51 ± 3	42 ± 3‡	−9 ± 3	54 ± 5	42 ± 4\$	−12 ± 2

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 ($n = 24$); androstenedione, 5.4 ± 0.5 nmol/l ($n = 24$);

DHEAS, 3.4 ± 0.3 μmol/l ($n = 24$); HMW adiponectin 15 ± 2 mg/l ($n = 21$); visfatin 31 ± 3 μg/l ($n = 21$); IMT 0.39 ± 0.02 mm ($n = 16$).

*Absence of significant differences between randomized subgroups at baseline.

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

body mass index (BMI) 23.7 ± 0.5 kg/m², 19.5–29.0 kg/m²; 5–12 years postmenarche; Table 1).

As reported⁴ the inclusion criteria were:

1 hyperinsulinaemia in a 2-h oral glucose tolerance test (OGTT), defined as peak serum insulin levels > 150 mU/l and/or mean serum insulin > 84 mU/l;

2 ovarian androgen excess, as defined by

- hirsutism (Ferriman–Gallwey score > 8), and;
- amenorrhoea (menses absent for > 3 months) or oligomenorrhoea (menstrual cycles > 45 days), and;
- elevated serum androstenedione, total testosterone or free androgen index [testosterone × 100/sex hormone-binding globulin (SHBG)], and;
- a 17-hydroxy-progesterone hyperresponse (17-OHP > 4.84 nmol/l) to a GnRH agonist (leuprolide acetate 500 μg s.c.).

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.

As reported,⁴ the exclusion criteria were: evidence of thyroid dysfunction, Cushing's syndrome or hyperprolactinaemia; glucose intolerance; personal history of diabetes mellitus; late-onset adrenal hyperplasia; anaemia; abnormal serum electrolytes; abnormal screening results for liver or kidney function; and abnormal echocardiogram.

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 assigned⁴ 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],

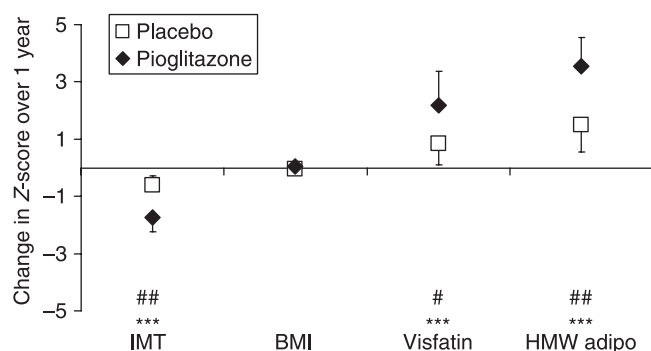


Fig. 1 Changes (0–1 year) in carotid intima media thickness, BMI, circulating visfatin and HMW adiponectin in hyperinsulinaemic women with androgen excess, treated with low-dose FluMet (21/28 days), a transdermal oestro-progestagen (21/28 days), and either placebo or pioglitazone (7.5 mg/day; 21/28 days). Changes are expressed as Z-scores, calculated by dividing the individual changes by the respective baseline SD in the study population. Plots represent means $\pm$ 95% CI. *** P < 0.001, for differences vs. baseline, in the total group. # P < 0.05; ## P < 0.01, for differences between subgroups. IMT, carotid intima media thickness; BMI, body mass index; HMW adipon, high molecular weight adiponectin.

after 6 months, and after 1 year (in week 3 of the cyclic on-treatment phase); here, only results spanning 1 year are reported.

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, dehydroepiandrosteronesulphate (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,⁴ 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

fit. VAT area was calculated by subtracting the organ areas from the intraperitoneal area.

Assays and statistics

Neutrophil and lymphocyte counts, serum glucose, insulin, testosterone, DHEAS, androstenedione, SHBG and CRP were measured as described;⁴ 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 $\pm$ 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 visceraally 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.¹¹

Hyperandrogenic women have high serum visfatin concentrations and have a high fraction of visceral fat,^{12,13} as our patients had at start of treatment. It is unclear how PioFluMet treatment amplified the baseline hypervisfatinaemia. One possibility is that the PioFluMet-associated rise in circulating visfatin is an epi-phenomenon of the reduction in visceral adiposity.¹⁴ The mechanisms underpinning the metabolic actions of thiazolidinediones are still controversial;^{8,9} at present, it is difficult to gauge to which extent the clinical benefits elicited by PioFluMet are mediated by hypervisfatinaemia.

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.

Acknowledgements

We thank Luis del Rio for assessments of visceral fat. LI is a Clinical Investigator of REDIMET, R676D (FIS, Instituto de Salud Carlos III, Madrid, Spain). AL-B is an Investigator of the Fund for Scientific Research 'Ramon y Cajal' (Ministry of Education and Science, Spain). FdZ is a Clinical Investigator of the Fund for Scientific Research (Flanders, Belgium).

References

- Ibáñez, L. & de Zegher, F. (2006) Low-dose flutamide–metformin therapy for hyperinsulinemic hyperandrogenism in non-obese adolescents and women. *Human Reproduction Update*, **12**, 243–252.
- Ibáñez, L., Valls, C. & de Zegher, F. (2006) Discontinuous low-dose flutamide–metformin plus an oral or a transdermal contraceptive in patients with hyperinsulinemic hyperandrogenism: normalizing effects on C-reactive protein, tumor necrosis factor- α and the neutrophil/lymphocyte ratio. *Human Reproduction*, **21**, 451–456.
- Gambineri, A., Patton, L., Vaccina, A., Cacciari, M., Morselli-Labate, A.M., Cavazza, C., Pagotto, U. & Pasquali, R. (2006) Treatment with flutamide, metformin, and their combination added to a hypocaloric diet in overweight-obese women with polycystic ovary syndrome: a randomized, 12-month, placebo-controlled study. *Journal of Clinical Endocrinology and Metabolism*, **91**, 3970–3980.
- Ibáñez, L., Lopez-Bermejo, A., del Rio, L., Enriquez, G., Valls, C. & de Zegher, F. (2007) Combined low-dose pioglitazone, flutamide, and metformin for women with androgen excess. *Journal of Clinical Endocrinology and Metabolism*, **92**, 1710–1714.
- Bodles, A.M., Banga, A., Rasouli, N., Ono, F., Kern, P.A. & Owens, R.J. (2006) Pioglitazone increases secretion of high-molecular-weight adiponectin from adipocytes. *American Journal of Physiology, Endocrinology and Metabolism*, **291**, E1100–E1105.
- Xu, A., Chan, K.W., Hoo, R.L., Wang, Y., Tan, K.C., Zhang, J., Chen, B., Lam, M.C., Tse, C., Cooper, G.J. & Lam, K.S. (2005) Testosterone selectively reduces the high molecular weight form of adiponectin by inhibiting its secretion from adipocytes. *Journal of Biological Chemistry*, **280**, 18073–18080.
- Fukuhara, A., Matsuda, M., Nishizawa, M., Segawa, K., Tanaka, M., Kishimoto, K., Matsuki, Y., Murakami, M., Ichisaka, T., Murakami, H., Watanabe, E., Takagi, T., Akiyoshi, M., Ohtsubo, T., Kihara, S., Yamashita, S., Makishima, M., Funahashi, T., Yamanaka, S., Hiramatsu, R., Matsuzawa, Y. & Shimomura, I. (2005) Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. *Science*, **307**, 426–430.
- Hammarstedt, A., Pihlajamäki, J., Rotter Sopasakis, V., Gogg, S., Jansson, P.A., Laakso, M. & Smith, U. (2006) Visfatin is an adipokine, but it is not regulated by thiazolidinediones. *Journal of Clinical Endocrinology and Metabolism*, **91**, 1181–1184.
- Haider, D.G., Mittermayer, F., Schaller, G., Artwohl, M., Baumgartner-Parzer, S.M., Prager, G., Roden, M. & Wolzt, M. (2006) Free fatty acids normalize a rosiglitazone-induced visfatin release. *American Journal of Physiology, Endocrinology and Metabolism*, **291**, E885–E890.
- Matthews, D.R., Hosker, J.P., Rudenski, A.S., Naylor, B.A., Treacher, D.F. & Turner, R.C. (1985) Homeostasis model assessment. insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*, **28**, 412–419.
- Glintborg, D., Frystyk, J., Højlund, K., Andersen, K.K., Henriksen, J.E., Hermann, A.P., Hagen, C., Flyvbjerg, A. & Andersen, M. (2007) Total and high molecular weight (HMW) adiponectin levels and measures of glucose and lipid metabolism following pioglitazone treatment in a randomized, placebo-controlled study in polycystic ovary syndrome. *Clinical Endocrinology*, doi: 10.1111/j.1365-2265.2007.03015.x.
- Tan, B.K., Chen, J., Digby, J.E., Keay, S.D., Kennedy, C.R. & Randeva, H.S. (2006) Increased visfatin messenger ribonucleic acid and protein levels in adipose tissue and adipocytes in women with polycystic ovary syndrome: parallel increase in plasma visfatin. *Journal of Clinical Endocrinology and Metabolism*, **91**, 5022–5028.
- Lord, J., Thomas, R., Fox, B., Acharya, U. & Wilkin, T. (2006) The central issue? Visceral fat mass is a good marker of insulin resistance and metabolic disturbance in women with polycystic ovary syndrome. *British Journal of Obstetrics and Gynaecology*, **113**, 1203–1209.
- Krzyzanowska, K., Mittermayer, F., Krugluger, W., Kopp, H.P. & Scherthaner, G. (2006) Increase in visfatin after weight loss induced by gastroplastic surgery. *Obesity*, **14**, 1886–1889.