

# Gender differences in non-glycemic responses to improved insulin sensitivity by pioglitazone treatment in patients with type 2 diabetes

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**Abstract** Excess cortisol and GH induce insulin resistance, a central feature of type 2 diabetes (T2D). To study whether the insulin sensitizer pioglitazone affects basal cortisol levels and the GH–IGF-I axis in patients with T2D. Forty-eight patients with T2D (men/women = 28:20, age  $61 \pm 1$  years, BMI  $31 \pm 0.6$  kg/m<sup>2</sup>) were treated for 26 weeks with pioglitazone 30–45 mg daily in addition to their preexisting therapy. Insulin, proinsulin, HbA<sub>1c</sub>, IGF-I, IGFBP-1, and basal cortisol were analyzed before and after treatment. Pioglitazone decreased proinsulin/insulin ratio and HbA<sub>1c</sub> decreased (HbA<sub>1c</sub> from  $7.8 \pm 0.2$  to  $6.6 \pm 0.2$  % in men and from  $7.6 \pm 0.2$  to  $6.1 \pm 0.2$  % in women,  $p < 0.001$  in both). There was a redistribution of fat but no change in waist circumference. IGF-I and adiponectin increased ( $p \leq 0.001$ ) in both genders. IGFBP-1 increased but significantly only for the whole group ( $p = 0.033$ ). Triglycerides decreased significantly in women only ( $p = 0.015$ ). Before treatment, women had lower basal cortisol ( $p = 0.045$ ). Basal cortisol increased in

women (from  $390 \pm 26$  to  $484 \pm 32$  nmol/L,  $p = 0.020$ ) but not in men and did not differ between genders at week 26.  $\Delta$ IGFBP-1 correlated with  $\Delta$ cortisol ( $r = 0.458$ ;  $p = 0.049$ ) and  $\Delta$ adiponectin ( $r = 0.600$ ;  $p = 0.005$ ) in women only. In addition to the known effect of improving insulin sensitivity, pioglitazone increased IGF-I regardless of gender and in women also increased basal cortisol. Increased IGF-I may contribute to improved insulin sensitivity after treatment. There seems to be gender differences in treatment responses to pioglitazone on lipid metabolism and basal cortisol, perhaps correcting different mechanisms of insulin resistance between genders.

**Keywords** Pioglitazone · Type 2 diabetes · Gender · Insulin-like growth factor · Cortisol

## Introduction

Insulin resistance in target tissues such as the adipose tissue, muscles, and liver is a well-characterized feature of type 2 diabetes (T2D) [1]. However, there are also pathological changes in the central hormone axes, with increased activity in the hypothalamus–pituitary–adrenal (HPA) axis and decreased activity in the remaining axes [2]. This may contribute to impaired metabolic control and development of various components of the metabolic syndrome including insulin resistance. Women generally develop cardiovascular disease and T2D at a later age than men; once insulin resistance has developed, however, they run the same risk of metabolic complications [3]. Much is still unknown about gender differences in the pathophysiology of insulin resistance and T2D, especially regarding the central hormone axes.

The hormone axes with the greatest impact on glucose metabolism are the growth hormone (GH)–insulin-like

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growth factor I (IGF-I) axis, and the HPA axis, which produces cortisol. IGF-I has effects highly comparable to those of insulin, increasing cellular uptake of glucose and free fatty acids (FFAs) as well as insulin sensitivity [4]. It is mainly produced in the liver but also locally in many tissues where it exerts metabolic effects [4]. IGF-I production is regulated by GH, insulin, and nutritional status whereby high levels of glucose and FFAs inhibit GH-IGF-I synthesis [5].

Insulin-like growth factor I bioavailability is mainly controlled by insulin-like growth factor binding protein 1 (IGFBP-1) [6], which helps to redistribute IGF-I from the circulation into tissues where it is active [7]. The production of IGFBP-1, also in the liver, is stimulated by factors such as glucagon, inflammatory cytokines, cortisol, and fasting [8]. Insulin acutely inhibits IGFBP-1 production, which allows it to be used as a marker of hepatic insulin sensitivity [9]. In states of insulin deficiency, such as type 1 diabetes, IGFBP-1 is elevated [10]. Patients with T2D can display either high IGF-I and low IGFBP-1 (due to insulin resistance with hyperinsulinemia) or low IGF-I and high IGFBP-1 (due to  $\beta$ -cell failure) [11].

Contrary to IGF-I, cortisol increases gluconeogenesis and insulin resistance [12]. Disturbances in HPA axis activation and feedback inhibition have been reported in obesity, insulin resistance, and T2D [2]. Given the phenotypic similarities between states of glucocorticoid excess and insulin resistance caused by diet-induced obesity, examining glucocorticoid levels in various states of insulin sensitivity is likely to be relevant to common obesity-related diseases like T2D and hypertension.

The purpose of the present study was primarily to study the interaction between the HPA and GH-IGF-I axes and (mainly hepatic) insulin sensitivity in men and women with T2D. To do so, we utilized pioglitazone, an insulin-sensitizing drug belonging to the family of thiazolidinediones (TZDs) and currently approved for the treatment for T2D. Largely, TZDs have effects opposite to those of cortisol [13]. Through the activation of peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ), TZDs improve insulin sensitivity in the liver (decreasing gluconeogenesis) and redistribute FFAs from insulin-resistant tissues such as the muscles and liver to the subcutaneous adipose tissue where they are less metabolically harmful [14]. Pilot studies on rats [15] have shown that pioglitazone increases IGF-I production and reduces the activity of the HPA axis. This study includes both men and women to elucidate whether gender differences may exist in the non-glycemic response to pioglitazone and the pathophysiology of T2D.

## Materials and methods

Sixty-six patients with T2D and secondary drug failure (see below) were screened for the study. Other entry criteria

were age 30–75 years and body mass index (BMI)  $>20$  kg/m<sup>2</sup>. Eight patients were ineligible due to either heart failure of NYHA class III-IV ( $n = 2$ ), severe renal dysfunction that demanded discontinuation of metformin therapy ( $n = 1$ ), ongoing medication with nonsteroidal anti-inflammatory drugs ( $n = 1$ ), cancer ( $n = 1$ ), proliferative retinopathy ( $n = 1$ ), severe hyperglycemic symptoms, or acute need of insulin therapy ( $n = 2$ ).

All patients included were on metformin and sulphonylurea (SU). Secondary drug failure was defined as HbA<sub>1c</sub>  $\geq 6.5$  % (Mono-S method) in the latest two measurements, separated by at least 8 weeks, during ongoing treatment with metformin  $>1,500$  mg/day and glibenclamide  $>7$  mg/day, glipizide  $>10$  mg/day, glimepiride  $>3$  mg/day or repaglinide  $>6$  mg/day for at least 3 months.

The study was approved by the local ethics committee and signed informed consent was obtained from all patients. The study was conducted in accordance with the ethical standards of the Declaration of Helsinki. Three patients were excluded during the study because of non-adherence to the protocol, and one dropped out due to side effects (vertigo, weight loss). These four patients declined to be followed up at the hospital; hence, they are not included in the analyses. Fifty-four patients completed the study, all of them Caucasian; results on metabolic parameters in these patients have been reported previously [16]. Sufficient serum only remained from 48 of the patients (28 men and 20 women) for the measurements of IGF-I, IGFBP-1, and cortisol. Thus, only analyses from these patients are included in this report.

## Study design

This was an interventional study that spanned 26 weeks. Blood sampling and the recording of body weight, cardiopulmonary symptoms, and other side effects were performed pre-interventionally, at intermediate visits after eight and 16 weeks, and at the end of the study. Blood samples for the analysis of HbA<sub>1c</sub>, lipid profile, serum insulin, proinsulin, IGF-I, IGFBP-1, and cortisol were taken pre-interventionally and at the end of the study. Blood tests were taken after an overnight fast, between 07:00 and 08:30 after 20-min rest in a supine position. Subjects received a prescription of 30 mg pioglitazone daily in addition to their preexisting therapy with metformin and sulphonylurea. After 16 weeks, the dose of pioglitazone was increased to 45 mg daily if HbA<sub>1c</sub> was not  $<6.5$  % and the therapy was well tolerated (Table 1).

## Assays

HbA<sub>1c</sub>, insulin, proinsulin, and adiponectin were analyzed using methods reported previously [16].

**Table 1** Subjects' medications (*n* on treatment) at baseline and at end of study

Drug	Men		Women	
	Baseline	End of study	Baseline	End of study
Metformin	28	28	20	20
Insulin secretagogue	28	28	20	20
Pioglitazone	0	28	0	20
ACE inhibitor	8	8	4	4
ARB	8	10	6	6
Aspirin 75–160 mg	8	8	6	6
β-blocker	10	10	5	5
Fibrate	9	9	2	1
Loop diuretic	4	5	2	3
Statins	18	18	10	10
Thiazide diuretic	5	5	3	3

Serum cortisol was analyzed at the Central Chemistry Laboratory at Karolinska University Hospital with chemoluminescence technique, using Roche Modular apparatus (Roche Diagnostics Scandinavia, Bromma, Sweden). The total coefficient of variation (CV) was 2.5 % at 544 nmol/L and 2.1 % at 855 nmol/L.

Serum IGF-I was determined by an in-house RIA after separation of IGFs from IGF-BPs by acid ethanol extraction and cryoprecipitation [17]. The detection level of the RIA was 3.0 mg/L. Cross-reactivity with IGF-BP-2 and IGF-BP-3 was less than 0.5 and 0.05 %, respectively. To minimize interference of remaining IGF-BPs, des(1–3) IGF-I was used as radioligand [17]. Serum levels of IGF-I decrease with age and are thus expressed as age-adjusted standard deviation (SD) score =  $[(10 \log IGF - I_{\text{observed}} + 0.00693 \times \text{age}) - 2.581] / 0.120$  [18]. The intra- and inter-assay CVs were 4 and 11 %, respectively.

Total serum IGF-BP-1 was also analyzed with RIA [19, 20] with a sensitivity of 3 μg/L. The intra- and inter-assay CVs were 3 and 10 %, respectively.

### Statistical analyses

All analyses were carried out using Statistica software version 10 (StatSoft, Tulsa, OH, USA). Data are expressed as mean ± SEM. Differences in normally distributed variables before and after intervention were tested by paired *t* test and in non-normally distributed variables by Wilcoxon rank test. Differences between group means were tested by unpaired *t* test or Mann–Whitney rank sum test, where appropriate. All tests were two-tailed and a *p* value of less than 0.05 was considered statistically significant. Changes in any given variable from baseline to week 26 are denoted Δ (delta). Correlations were analyzed using product–moment correlations.

## Results

### Subject characteristics

Patients' characteristics at baseline are seen in Table 2. There was no significant difference between the men and women in the study in age, duration of disease, BMI, fasting plasma glucose (FPG), or proinsulin/insulin ratio at baseline. Hormone levels at baseline and after treatment are summarized in Table 2.

### Pioglitazone improved metabolic control and beta-cell function

As previously reported, pioglitazone improved metabolic control as measured by the levels of HbA<sub>1c</sub>, despite an increase in weight, BMI, hip circumference, and thereby a decreased waist–hip ratio in both genders [16]. ΔHbA<sub>1c</sub> was predicted by FPG at baseline ( $r = 0.315$ ,  $p = 0.029$ ) in all subjects when pooled, but not in men or women individually. Triglycerides decreased only in women. Except for triglycerides, there were no significant changes in lipid levels or waist circumference when the groups were pooled or analyzed separately (see Table 2).

There was no change in serum insulin levels, but proinsulin and the proinsulin/insulin ratio decreased significantly. Adiponectin increased in both men and women; levels did not differ between the gender groups before or after treatment (see Table 3).

### Serum IGF-I increased significantly

Insulin-like growth factor I increased significantly in both women and men ( $p < 0.001$  in both; Fig. 1a). There were no gender differences in IGF-I levels before or during treatment, nor in the magnitude of the increase.

### Serum IGF-BP-1 increased significantly without decrease in serum insulin

When all subjects were analyzed together, IGF-BP-1 increased during pioglitazone treatment ( $p = 0.033$ ). However, when men and women were analyzed separately, neither increase reached statistical significance (Fig. 1b). The levels did not differ between the genders before or during treatment.

### Gender differences in cortisol

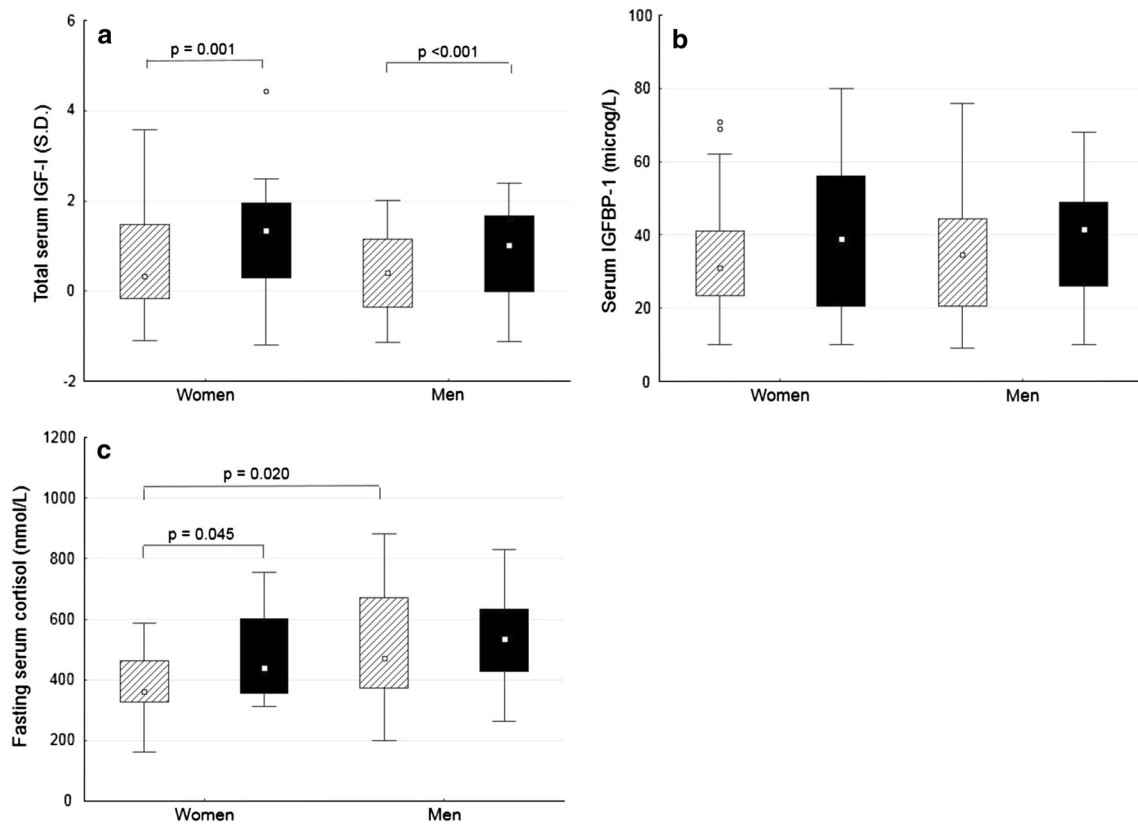
At baseline, men had higher serum cortisol ( $p = 0.045$ ; Fig. 1c). Serum cortisol increased among women ( $p = 0.020$ ), whereas it was unchanged among men. After 26 weeks of treatment, there was no longer a difference between the genders in serum cortisol.

**Table 2** Subject characteristics at baseline and after 26 weeks of pioglitazone treatment (mean (range) for age and duration; mean  $\pm$  SEM for remaining variables)

	All subjects				Men		Women		Men versus women			
	Baseline	Week 26	<i>p</i>	Baseline	Week 26	<i>p</i>	Baseline	Week 26	<i>p</i>	<i>p</i> baseline	<i>p</i> week 26	<i>p</i> $\Delta$
Age at inclusion (years)	61 (45–76)	na	na	60 (50–71)	na	na	62 (45–76)	na	na	0.017	na	na
Duration of diabetes (years)	11 (3–30)	na	na	10 (3–30)	na	na	13 (4–21)	na	na	0.096	na	na
Fasting <i>p</i> -glucose (mmol/L)	10.5 $\pm$ 0.3	na	na	10.5 $\pm$ 0.4	na	na	10.6 $\pm$ 0.5	na	na	0.934	na	na
HbA <sub>1c</sub> [NGSP % (IFCC mmol/mol)]	8.6 $\pm$ 2.8 (70 $\pm$ 7)	7.3 $\pm$ 2.8 (56 $\pm$ 7)	<0.001	8.6 $\pm$ 2.7 (71 $\pm$ 6)	7.6 $\pm$ 2.7 (58 $\pm$ 6)	<0.001	8.6 $\pm$ 2.7 (70 $\pm$ 6)	7.0 $\pm$ 2.7 (53 $\pm$ 6)	<0.001	0.536	0.145	0.088
Weight (kg)	90.2 $\pm$ 2.2	94.1 $\pm$ 2.3	<0.001	95.9 $\pm$ 2.9	99.9 $\pm$ 3.1	<0.001	82.2 $\pm$ 2.4	86.0 $\pm$ 2.7	<0.001	0.001	0.002	0.958
BMI (kg/m <sup>2</sup> )	31.0 $\pm$ 0.6	32.3 $\pm$ 0.6	<0.001	30.5 $\pm$ 0.8	31.8 $\pm$ 0.8	<0.001	31.6 $\pm$ 0.8	33.0 $\pm$ 0.9	<0.001	0.395	0.360	0.594
Waist circumference (cm)	111 $\pm$ 2	112 $\pm$ 2	0.132	111 $\pm$ 2	112 $\pm$ 2	0.081	110 $\pm$ 3	111 $\pm$ 3	0.544	0.719	0.649	0.925
Hip circumference (cm)	107 $\pm$ 1	111 $\pm$ 1	<0.001	106 $\pm$ 1	109 $\pm$ 2	<0.001	108 $\pm$ 2	114 $\pm$ 2	0.001	0.469	0.069	0.191
Waist/hip ratio	1.04 $\pm$ 0.01	1.00 $\pm$ 0.01	0.001	1.05 $\pm$ 0.01	1.03 $\pm$ 0.01	0.004	1.02 $\pm$ 0.02	0.97 $\pm$ 0.02	0.022	0.279	0.003	0.458

**Table 3** Hormone and lipid levels before and during pioglitazone treatment (mean  $\pm$  SEM)

	All subjects				Men		Women		Men versus women			
	Baseline	Week 26	<i>p</i>	Baseline	Week 26	<i>p</i>	Baseline	Week 26	<i>p</i>	<i>p</i> baseline	<i>p</i> week 26	<i>p</i> $\Delta$
Insulin (pmol/L)	8.2 $\pm$ 0.7	9.4 $\pm$ 1.1	0.784	7.5 $\pm$ 0.9	9.7 $\pm$ 1.8	0.156	9.2 $\pm$ 1.2	8.9 $\pm$ 1.1	0.171	0.196	0.892	0.061
Proinsulin (pmol/L)	44.4 $\pm$ 5.7	30.4 $\pm$ 4.0	<0.001	45.1 $\pm$ 7.7	31.0 $\pm$ 4.5	0.006	43.3 $\pm$ 8.7	29.7 $\pm$ 7.5	<0.001	0.722	0.439	0.236
Ratio proinsulin/insulin	5.4 $\pm$ 0.5	3.4 $\pm$ 0.3	<0.001	6.2 $\pm$ 0.8	3.7 $\pm$ 0.4	<0.001	4.3 $\pm$ 2.2	3.0 $\pm$ 0.4	<0.001	0.092	0.208	0.107
Cortisol (nmol/L)	451 $\pm$ 24	518 $\pm$ 22	0.017	493 $\pm$ 36	542 $\pm$ 29	0.264	390 $\pm$ 26	484 $\pm$ 32	0.020	0.045	0.192	0.823
IGF-I (SD)	0.5 $\pm$ 0.2	1.0 $\pm$ 0.2	<0.001	0.4 $\pm$ 0.2	0.9 $\pm$ 0.2	0.001	0.6 $\pm$ 0.3	1.2 $\pm$ 0.3	<0.001	0.510	0.331	0.975
IGFBP-1 ( $\mu$ g/L)	34.5 $\pm$ 2.6	39.4 $\pm$ 2.6	0.033	34.9 $\pm$ 3.5	38.9 $\pm$ 2.9	0.052	34.0 $\pm$ 4.1	40.0 $\pm$ 4.9	0.295	0.749	0.892	0.810
Adiponectin ( $\mu$ g/ml)	6.2 $\pm$ 0.4	13.2 $\pm$ 0.9	<0.001	5.7 $\pm$ 0.4	12.2 $\pm$ 0.8	<0.001	6.9 $\pm$ 0.8	14.6 $\pm$ 1.7	<0.001	0.130	0.169	0.794
Cholesterol (mmol/L)	4.7 $\pm$ 0.1	4.7 $\pm$ 0.1	0.744	4.5 $\pm$ 0.1	4.6 $\pm$ 0.2	0.893	4.8 $\pm$ 0.2	4.8 $\pm$ 0.2	0.888	0.206	0.380	0.811
HDL (mmol/L)	1.1 $\pm$ 0.0	1.1 $\pm$ 0.0	0.165	1.1 $\pm$ 0.0	1.1 $\pm$ 0.1	0.478	$\pm$ 0.1	1.2 $\pm$ 0.1	0.191	0.601	0.310	0.409
LDL (mmol/L)	2.8 $\pm$ 0.1	2.8 $\pm$ 0.1	0.510	2.7 $\pm$ 0.1	2.7 $\pm$ 0.1	0.704	2.9 $\pm$ 0.1	2.9 $\pm$ 0.2	0.619	0.566	0.515	0.608
TG (mmol/L)	1.9 $\pm$ 0.1	1.6 $\pm$ 0.1	0.017	1.7 $\pm$ 0.2	1.6 $\pm$ 0.2	0.319	2.1 $\pm$ 0.2	1.7 $\pm$ 0.2	0.015	0.188	0.631	0.129



**Fig. 1** Fasting total serum IGF-I (a), IGFBP-1 (b), and cortisol (c) at baseline and after 26 weeks of pioglitazone treatment. *Striped bars* before treatment, *black bars* during treatment, *circles* outliers

## Correlations

Neither baseline nor  $\Delta$ cortisol correlated with  $\Delta$ HbA<sub>1c</sub>. Neither the increase in weight nor the decrease in WHR correlated with fasting cortisol or IGF-I in either group.

However,  $\Delta$ cortisol correlated positively with  $\Delta$ IGFBP-1 ( $r = 0.458$ ,  $p = 0.049$ ) in women only. Finally,  $\Delta$ IGFBP-1 correlated positively with  $\Delta$ adiponectin ( $r = 0.600$ ,  $p = 0.005$ ) in women only.

## Discussion

In this study of men and women with T2D, 26 weeks of treatment with pioglitazone decreased HbA<sub>1c</sub> and reduced the proinsulin/insulin quotient indicating reduced  $\beta$ -cell stress [21]. Both IGF-I and IGFBP-1 increased, without any clear difference between the genders. As IGF-I contributes to approximately 10 % of total insulin sensitivity [22], its increase may explain a small portion of the improved insulin sensitivity following pioglitazone treatment. However, it is more likely that the increase is a marker of increased GH secondary to improved lipid metabolism. In a similar study on non-diabetic women with

PCOS and insulin resistance, 16 weeks of pioglitazone treatment resulted in increased GH, IGF-I, and adiponectin; however, no effect was seen on 24-h mean cortisol levels or urinary cortisol metabolites [23]. Increased IGF-I, IGFBP-1, and adiponectin are the indicators of improved insulin sensitivity [22, 24].

Triglyceride levels decreased only in women, while insulin was unaffected and proinsulin decreased in both gender groups. This indicates that only women experienced improved insulin sensitivity of the adipose tissue, the result of which is reduced lipolysis, and thereby lower FFAs. It is well known that TZDs reduce FFAs [14], which in turn would result in increased GH levels and secondarily increased IGF-I [5]. IGF-I levels increased toward the age-adjusted mean, not to supernormal levels, also indicating a normalization of a deranged hormonal milieu. However, this occurred in both groups despite only women displaying reduced triglycerides. FFAs were not measured, so it cannot be excluded that these also decreased in men despite the other markers of lipolysis remaining unchanged.

Insulin-like growth factor I binds to its own receptor (IGF-IR), the insulin receptor at supraphysiological levels, and also to hybrid receptors composed of subunits of both the insulin and IGF-I receptors [4]. Hybrid receptors are

expressed in many tissues including adipocytes and the liver [4]. They can bind both insulin and IGF-I, but bind the latter with higher affinity [25]. Hybrid receptor expression is increased in skeletal muscle and adipose tissue in T2D [25], and is negatively correlated with insulin sensitivity [26]. Hyperinsulinemia in insulin resistance is also associated with decreased expression of regular insulin receptors, allowing increased hybrid receptor expression [27]. Based on this, it is plausible that increased insulin sensitivity during pioglitazone treatment was associated with reduced expression of these hybrid receptors to more normal levels, as insulin's ability to activate its own receptor increased. Hence, improved insulin sensitivity and reduced expression of hybrid receptors in the liver may have contributed to the increase in IGFBP-1.

The increase in IGFBP-1 was significant when both genders were pooled but not when they were analyzed separately, probably due to small sample size. As noted previously, IGFBP-1 is a marker of hepatic insulin sensitivity [9], so the increase in IGFBP-1 most likely reflects that this improved. Pioglitazone is known to reduce hepatosteatosis, which would also contribute to this improvement [14].

Compared to a population of healthy controls, adiponectin increased from subnormal to normal levels in both genders after treatment [28]. This reflects the strong correlation between adiponectin and insulin sensitivity [24]. However,  $\Delta$ IGFBP-1 correlated with  $\Delta$ adiponectin only in women. Gender differences in this correlation have previously been reported in healthy controls by our group [28]. Adiponectin is a marker of adipocyte differentiation [29], which pioglitazone is known to increase [30], and is negatively correlated with WHR in both men and women [31]. Greater improvement in adipose tissue metabolism after treatment could explain the stronger correlation between changes in IGFBP-1 and adiponectin in women.

Hypercortisolism is known to induce insulin resistance by a number of mechanisms, leading to increased gluconeogenesis, decreased glucose uptake, and increased lipolysis [12]. Given this fact, the observed improvement of glycemic control in women, in the face of increased cortisol levels, may seem paradoxical. However, before pioglitazone treatment, cortisol levels were lower in women. Gender differences were shown in one previous study, where healthy women had lower endogenous cortisol levels than men even after correction for body surface area [32]. After treatment in our study, the men's levels remained unchanged, while the women's rose to become equal to those in the male group, but within the bounds of normal levels. The female patients showed improved HbA<sub>1c</sub> despite the rise in cortisol, without any correlation between the two. Likewise, hypercortisolemia is linked with dyslipidemia [33], whereas TG levels improved

significantly in this study among women, but not among men. This suggests that the improved metabolic parameters seen in female patients are due to factors other than cortisol after pioglitazone treatment, for example, FGF-21 which is known to regulate PPAR $\gamma$  activity and the antidiabetic actions of TZDs [34].

This study does not provide an explanation for the increase in basal cortisol among females. Basal cortisol is determined by the activity of the HPA axis, peripheral synthesis from inactive cortisol via the enzyme 11 $\beta$ -hydroxysteroid dehydrogenase type 1, peripheral metabolism, and excretion. Studies on rats [15] and patients with Cushing's disease [35] suggest that TZDs reduce the activity of the HPA axis. Like FFAs, corticotropin-releasing hormone (CRH) has an inhibiting effect on GH [36]. CRH effects were reduced after treatment with rosiglitazone, another TZD, in one animal study [15]. Reduced CRH would increase GH and IGF-I, as seen in both genders in this study. Another possibility is that changes in the GH–IGF-I axis influence HPA axis activity in men, preventing the increase in cortisol that occurred in women. Such an effect has been seen for growth hormone–releasing hormone, which inhibits the HPA axis in men selectively [37]. IGF-I is unlikely to mediate such an effect directly, as IGF-I administration has not shown any effect on the ACTH or cortisol response to CRH [38]. However, the exact cause of the increase is beyond the scope of this study.

As with adiponectin,  $\Delta$ IGFBP-1 correlated with  $\Delta$ cortisol in women alone. Cortisol stimulates IGFBP1 gene transcription [39] and cortisol infusion has been shown to increase IGFBP-1 secretion [40]. However, as an increase in cortisol only occurred in women, this is unlikely to explain the increase in IGFBP-1 which was not gender specific.

In postmenopausal women, treatment with rosiglitazone decreases bone density and increases the risk of fractures [41]. This effect has not been observed in men. Exogenous glucocorticoid treatment is associated with bone loss and increased fracture risk [42], just as in women on TZD treatment. It is possible that the increase in cortisol, seen in women in this study, contributes to the increased fracture risk. Glucocorticoids inhibit transcriptional activation of osteocalcin [12], which has been suggested both to be a candidate factor underlying glucocorticoid-induced osteoporosis [43] and to contribute to the metabolic derangements in glucocorticoid treatment [12]. Hence, measurement of osteocalcin may be of interest in future studies on the cause of increased fracture risk with TZD treatment.

In summary, to date, little is known about gender differences in the pathophysiology of T2D. The results of this hypothesis-generating study indicate that differences exist in hormones that contribute to glucose metabolism in T2D

patients, and that they differ in their response to pioglitazone treatment. The strongest effect was seen on the GH–IGF-I axis, where no gender difference was noted, and in the fasting serum cortisol level and triglycerides, where the gender differences were clear. This suggests that the GH–IGF-I axis is important to the effect of pioglitazone. More research is needed to determine the cause of the observed gender differences in the HPA axis, and the role of the greater improvement in adipose tissue metabolism in women.

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**Conflict of interest** none.

## References

- Stumvoll M, Gerich J (2001) Clinical features of insulin resistance and beta cell dysfunction and the relationship to type 2 diabetes. *Clin Lab Med* 21:31–51
- Bjorntorp P, Holm G, Rosmond R (1999) Hypothalamic arousal, insulin resistance and Type 2 diabetes mellitus. *Diabet Med* 16:373–383
- Barrett-Connor E (1997) Sex differences in coronary heart disease: why are women so superior? The 1995 Ancel Keys Lecture. *Circulation* 95:252–264
- Rajpathak SN, Gunter MJ, Wylie-Rosett J et al (2009) The role of insulin-like growth factor-I and its binding proteins in glucose homeostasis and type 2 diabetes. *Diabetes Metab Res Rev* 25:3–12
- Moller N, Vendelbo MH, Kampmann U et al (2009) Growth hormone and protein metabolism. *Clin Nutr* 28:597–603
- Baxter RC (1997) Insulin-like growth factor (IGF) binding proteins: the role of serum IGF-BPs in regulating IGF availability. *Acta paediatr Scand Suppl* 372:107–114; discussion 115
- Young SCJ, Miles MV, Clemmons DR (1992) Determination of the pharmacokinetic profiles of insulin-like growth factor binding proteins-1 and -2 in rats. *Endocrinology* 131:1867–1873
- Wheatcroft SB, Kearney MT (2009) IGF-dependent and IGF-independent actions of IGF-binding protein-1 and -2: implications for metabolic homeostasis. *Trends Endocrinol Metab* 20:153–162
- Kotronen A, Lewitt M, Hall K et al (2008) Insulin-like growth factor binding protein 1 as a novel specific marker of hepatic insulin sensitivity. *J Clin Endocrinol Metab* 93:4867–4872
- Brismar K, Gutniak M, Pova G et al (1988) Insulin regulates the 35 kDa IGF binding protein in patients with diabetes mellitus. *J Endocrinol Invest* 11:599–602
- Heald AH, Cruickshank JK, Riste LK et al (2001) Close relation of fasting insulin-like growth factor binding protein-1 (IGFBP-1) with glucose tolerance and cardiovascular risk in two populations. *Diabetologia* 44:333–339
- Ferris HA, Kahn CR (2012) New mechanisms of glucocorticoid-induced insulin resistance: make no bones about it. *J Clin Invest* 122:3854–3857
- Wake DJ, Stimson RH, Tan GD et al (2007) Effects of peroxisome proliferator-activated receptor-(alpha) and -(gamma) agonists on 11(beta)-hydroxysteroid dehydrogenase type 1 in subcutaneous adipose tissue in men. *J Clin Endocrinol Metab* 92:1848–1856
- Yki-Jarvinen H (2004) Thiazolidinediones. *N Engl J Med* 351:1106–1118+1158
- Heaney AP, Fernando M, Yong WH, Melmed S (2002) Functional PPAR-(gamma) receptor is a novel therapeutic target for ACTH-secreting pituitary adenomas. *Nat Med* 8:1281–1287
- Dorkhan M, Magnusson M, Frid A et al (2006) Glycaemic and nonglycaemic effects of pioglitazone in triple oral therapy of patients with type 2 diabetes. *J Intern Med* 260:125–133
- Bang P, Eriksson U, Sara V et al (1991) Comparison of acid ethanol extraction and acid gel filtration prior to IGF-I and IGF-II radioimmunoassays: improvement of determinations in acid ethanol extracts by the use of truncated IGF-I as radioligand. *Acta Endocrinol* 124:620–629
- Hilding A, Brismar K, Degerblad M et al (1995) Altered relation between circulating levels of insulin-like growth factor-binding protein-1 and insulin in growth hormone-deficient patients and insulin-dependent diabetic patients compared to that in healthy subjects. *J Clin Endocrinol Metab* 80:2646–2652
- Pova G, Roovete A, Hall K (1984) Cross-reaction of serum somatomedin-binding protein in a radioimmunoassay developed for somatomedin-binding protein isolated from human amniotic fluid. *Acta Endocrinol* 107:563–570
- Soderberg S, Ahren B, Eliasson M et al (2001) Circulating IGF binding protein-1 is inversely associated with leptin in non-obese men and obese postmenopausal women. *Eur J Endocrinol* 144:283–290
- Hellenius MLB, Brismar KE, Berglund BH, De Faire UH (1995) Effects on glucose tolerance, insulin secretion, insulin-like growth factor 1 and its binding protein, IGFBP-1, in a randomized controlled diet and exercise study in healthy, middle-aged men. *J Intern Med* 238:121–130
- Sesti G, Sciacqua A, Cardellini M et al (2005) Plasma concentration of IGF-I is independently associated with insulin sensitivity in subjects with different degrees of glucose tolerance. *Diabetes Care* 28:120–125
- Glintborg D, Hermann AP, Hagen C et al (2009) A randomized placebo-controlled study on the effects of pioglitazone on cortisol metabolism in polycystic ovary syndrome. *Fertil Steril* 91:842–850
- Weyer C, Funahashi T, Tanaka S et al (2001) Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab* 86:1930–1935
- Sesti G, Federici M, Lauro D et al (2001) Molecular mechanism of insulin resistance in type 2 diabetes mellitus: role of the insulin receptor variant forms. *Diabetes Metab Res Rev* 17:363–373
- Federici M, Porzio O, Zucaro L et al (1997) Increased abundance of insulin/IGF-I hybrid receptors in adipose tissue from NIDDM patients. *Mol Cell Endocrinol* 135:41–47
- Federici M, Lauro D, D'Adamo M et al (1998) Expression of insulin/IGF-I hybrid receptors is increased in skeletal muscle of patients with chronic primary hyperinsulinemia. *Diabetes* 47:87–92
- Andreasson AN, Uden AL, Elovsson S, Brismar K (2012) Leptin and adiponectin: distribution and associations with cardiovascular risk factors in men and women of the general population. *Am J Hum Biol* 24:595–601
- Korner A, Wabitsch M, Seidel B et al (2005) Adiponectin expression in humans is dependent on differentiation of adipocytes and down-regulated by humoral serum components of high molecular weight. *Biochem Biophys Res Commun* 337:540–550
- Sharma AM, Staels B (2007) Review: peroxisome proliferator-activated receptor (gamma) and adipose tissue—understanding

- obesity-related changes in regulation of lipid and glucose metabolism. *J Clin Endocrinol Metab* 92:386–395
31. Vilarrasa N, Vendrell J, Maravall J et al (2005) Distribution and determinants of adiponectin, resistin and ghrelin in a randomly selected healthy population. *Clin Endocrinol* 63:329–335
  32. Vierhapper H, Nowotny P, Waldhausl W (1998) Sex-specific differences in cortisol production rates in humans. *Metabolism* 47:974–976
  33. Lonn L, Kvist H, Ernest I, Sjostrom L (1994) Changes in body composition and adipose tissue distribution after treatment of women with Cushing's syndrome. *Metabolism* 43:1517–1522
  34. Dutchak PA, Katafuchi T, Bookout AL et al (2012) Fibroblast growth factor-21 regulates PPAR( $\gamma$ ) activity and the anti-diabetic actions of thiazolidinediones. *Cell* 148:556–567
  35. Ambrosi B, Dall'Asta C, Cannavo S et al (2004) Effects of chronic administration of PPAR-( $\gamma$ ) ligand rosiglitazone in Cushing's disease. *Eur J Endocrinol* 151:173–178
  36. Tsigos C, Chrousos GP (2002) Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. *J Psychosom Res* 53:865–871
  37. Steiger A (2007) Neurochemical regulation of sleep. *J Psychiatr Res* 41:537–552
  38. Gianotti L, Ramunni J, Lanfranco F et al (2001) Recombinant human IGF-I does not modify the ACTH and cortisol responses to hCRH and hexarelin, a peptidyl GH secretagogue, in humans. *J Endocrinol Invest* 24:67–71
  39. Terzolo M, Reimondo G, Bovio S, Angeli A (2004) Subclinical Cushing's syndrome. *Pituitary* 7:217–223
  40. Conover CA, Divertie GD, Lee PDK (1993) Cortisol increases plasma insulin-like growth factor binding protein-1 in humans. *Acta Endocrinol* 128:140–143
  41. Schwartz AV, Sellmeyer DE, Vittinghoff E et al (2006) Thiazolidinedione use and bone loss in older diabetic adults. *J Clin Endocrinol Metab* 91:3349–3354
  42. Gourlay M, Franceschini N, Sheyn Y (2007) Prevention and treatment strategies for glucocorticoid-induced osteoporotic fractures. *Clin Rheumatol* 26:144–153
  43. Heinrichs AAJ, Bortell R, Rahman S et al (1993) Identification of multiple glucocorticoid receptor binding sites in the rat osteocalcin gene promoter. *Biochemistry* 32:11436–11444