CLINICAL STUDY

Pioglitazone improves insulin resistance and decreases blood pressure in adult patients with congenital adrenal hyperplasia

Jeanne Margot Kroese^{1,2}, Christiaan F Mooij^{1,3}, Marinette van der Graaf⁴, Ad R M M Hermus¹ and Cees J Tack²

Departments of ¹Endocrinology, ²General Internal Medicine, ³Paediatric Endocrinology and ⁴Radiology, Radboud University Nijmegen Medical Centre, PO Box 9101, 6500 HB Nijmegen, The Netherlands

(Correspondence should be addressed to C F Mooij at Department of Paediatric Endocrinology, Radboud University Nijmegen Medical Centre; Email: c.mooij@cukz.umcn.nl)

Abstract

Context: Patients with congenital adrenal hyperplasia (CAH) are chronically treated with supraphysiological doses of glucocorticoids, which are known to induce insulin resistance. Thiazolidinediones might reverse this effect and improve insulin sensitivity.

Objectives: To assess insulin sensitivity in CAH patients and the effect of pioglitazone treatment on insulin sensitivity in CAH patients. Secondary objectives were the effects of treatment with pioglitazone on blood pressure, body fat distribution, lipid, and steroid profiles.

Design: Randomized placebo controlled crossover trial.

Participants: Twelve CAH patients and 12 body mass and age-matched control subjects.

Intervention: Sixteen-week treatment with pioglitazone (45 mg/day) or placebo.

Main outcome measure: Insulin sensitivity measured by euglycemic clamp and oral glucose tolerance test. Further measures were 24-h blood pressure profiles, body fat distribution measured by magnetic resonance imaging, dual energy x-ray absorptiometry (DEXA) and bioimpedance procedures, liver fat by magnetic resonance spectroscopy, lipid, and steroid profiles.

Results: CAH patients were insulin resistant compared with healthy controls. Treatment with pioglitazone significantly improved insulin sensitivity in CAH patients (glucose infusion rate (GIR) from 28.5 ± 11.6 to 38.9 ± 11.0 $\mu\text{mol/kg per min}$, $P=0.000$, GIR in controls 46.2 ± 23.4 $\mu\text{mol/kg per min}$, $P<0.05$ versus CAH). Treatment with pioglitazone decreased blood pressure (systolic: 124.0 ± 13.6 vs 127.0 ± 14.9 mmHg, $P<0.001$, diastolic: 72.8 ± 11.5 vs 77.4 ± 12.6 mmHg, $P<0.001$). No changes in body fat distribution, lipid, and steroid profiles were observed.

Conclusions: CAH patients are insulin resistant compared with matched control subjects. Treatment with pioglitazone improves insulin sensitivity and decreases blood pressure in CAH patients.

European Journal of Endocrinology 161 887–894

Introduction

Congenital adrenal hyperplasia (CAH) is an autosomal recessive condition, which is in more than 95% of the cases caused by a mutation in the gene for 21-hydroxylase (1). This defect results in increased secretion of ACTH, adrenal hyperplasia and increased production of androgens, but glucocorticoid deficiency. Current treatment comprises glucocorticoid (and, if necessary, mineralocorticoid) administration to prevent adrenal crises and suppress the abnormal secretion of androgens. However, because glucocorticoids are given in slightly supraphysiological doses and the therapeutic range for glucocorticoids is narrow, patients are at risk of developing iatrogenic Cushing's syndrome (1).

Glucocorticoids induce insulin resistance in humans (2), and may precipitate diabetes in susceptible subjects. Chronic exogenous or endogenous oversupply with glucocorticoids is associated with changes in fat

distribution, typically toward more central/visceral fat. Whether the observed insulin resistance is related to these changes in body fat distribution is not known.

The use of supraphysiological doses of glucocorticoids and androgen excess in CAH patients are associated with the development of an unfavorable cardiovascular risk profile (3). Data on the prevalence of cardiovascular morbidity or life expectancy in CAH patients are not yet available. In this respect, it is of interest that a recent population-based study in Sweden has showed that patients with Addison's disease have a clearly increased mortality rate. The excess mortality in both males and females was for a major part caused by cardiovascular disease. The authors of this study suggested that the increased cardiovascular mortality in Addison's patients is caused by excess glucocorticoid exposure (4).

Of the few studies that have explored insulin sensitivity in CAH, most have concluded that CAH patients are indeed characterized by insulin resistance

(5–11). Many different methods have been used to assess insulin sensitivity, but studies using the euglycemic hyperinsulinemic clamp method, the gold standard to assess insulin sensitivity, are lacking. It has also been reported that CAH patients are at risk of developing other components of the metabolic syndrome (3, 12, 13).

CAH patients are characterized by insulin resistance and have an unfavorable cardiovascular risk profile, so it might be a rational option to improve their insulin sensitivity with insulin sensitizers. Improving their cardiovascular risk profile might also improve their life expectancy. Thiazolidinediones (TZDs) improve insulin sensitivity in several conditions associated with insulin resistance. The improvement of insulin sensitivity is associated with beneficial changes in several parameters, like lipids, blood pressure, and vascular function, and changes in inflammatory markers (14, 15). Therefore, TZDs might be a rational therapeutic option in glucocorticoid-induced insulin resistance, as has been suggested by earlier studies (16, 17). Such improvement in insulin sensitivity might have a twofold benefit: first, glucose tolerance in patients with limited β -cell function will improve and hence the chance to develop overt type 2 diabetes will decrease. Secondly, cardiovascular risk factors associated with insulin resistance will improve (18).

In the present study, we tested the hypotheses that CAH patients who are chronically treated with glucocorticoids are insulin resistant and that treatment with pioglitazone improves insulin sensitivity in this group. Insulin sensitivity was assessed using the euglycemic clamp procedure. We also measured the effect of pioglitazone treatment on body fat distribution, including liver fat content, blood pressure, and lipid profile.

Materials and methods

Patients

Adult subjects with biochemically and genetically proven CAH on a stable corticosteroid replacement dose for 3 months were included in the study. Characteristics of the study population are shown in Table 1. Gender, phenotype, results of mutation analysis, medication, and mean salivary androstenedione levels of the study population are shown in Table 2. Exclusion criteria were as follows: age <18 years, inability to give informed consent, significant cardiovascular disease (defined as myocardial infarction or stroke 6 months preceding the study), significant renal disease (defined as a glomerular filtration rate (GFR) <30 ml/min), significant liver disease (defined as alanine aminotransferase and aspartate aminotransferase levels of more than three times the upper limit of normal), and mental disease. All patients were fully

Table 1 Characteristics of patients and controls.

	CAH patients	Control subjects
Gender (M/F)	5/7	5/7
Age (years)	35.7 $\pm$ 8.9	37.6 $\pm$ 6.5
Height (m)	1.64 $\pm$ 0.11	1.73 $\pm$ 0.09
Weight (kg)	72.6 $\pm$ 15.1	79.5 $\pm$ 14.3
BMI (kg/m ²)	26.9 $\pm$ 4.7	26.6 $\pm$ 5.1
Waist (cm)	87.1 $\pm$ 10.7	
Hip (cm)	101.3 $\pm$ 10.5	
Waist/hip	0.86 $\pm$ 0.09	
Systolic blood pressure (mmHg)		
Supine	134 $\pm$ 14	124 $\pm$ 11
Upright	125 $\pm$ 12	
Diastolic blood pressure (mmHg)		
Supine	83 $\pm$ 8	78 $\pm$ 12
Upright	87 $\pm$ 11	
Heart rate (bpm)		
Supine	68 $\pm$ 6	70 $\pm$ 10
Upright	77 $\pm$ 8	

M, male; F, female; bpm, beats per minute.

informed about the aim and design of the study and all the methods involved. They consented with the study protocol which was approved by the institutional review board of the Radboud University Nijmegen Medical Centre.

Insulin sensitivity in CAH patients treated with glucocorticoids was compared to insulin sensitivity measured in a group of body mass index (BMI) and age-matched normal subjects. These subjects were selected in a case–control design from a larger cohort earlier described (19). Characteristics of the control group are shown in Table 1.

Methods

At screening, all patients underwent a full physical examination and electrocardiography. Blood was drawn for determination of liver function and renal function. To estimate GFR, the MDRD–GFR equation was used (20).

After a 4-week run-in phase, patients were randomized to treatment with either placebo for 16 weeks, followed by pioglitazone (45 mg/day) for 16 weeks, or treatment with pioglitazone for 16 weeks, followed by placebo for 16 weeks in a randomized crossover design. Patients visited the clinic every 2 months for medication checks, adverse events, and measurement of weight, blood pressure, and edema formation. Safety measures included assessment of weight, blood pressure, and edema. At the end of the respective study periods, fasting glucose, lipid, and hormone levels (insulin, ACTH, cortisol, aldosterone, renin, 17-OH progesterone and androstenedione in serum, and 17-OH progesterone and androstenedione in saliva in the morning, afternoon, and evening) were assessed and insulin sensitivity was measured using a euglycemic hyperinsulinemic (steady-state plasma insulin level

Table 2 Age, gender, phenotype, mutation analysis, medication, and mean salivary androstenedione levels in 12 included congenital adrenal hyperplasia (CAH) patients.

P	Age (years)	Gender	Phenotype ^a	Allele 1 ^b	Allele 2 ^b	BMI (kg/m ²)	Daily glucocorticoid therapy	Daily mineralocorticoid therapy ^c	Mean salivary androstenedione levels (nmol/l) ^d
1	28	F	Nonclassical	c.841G>T (p.Val281Leu)	c.841G>T (p.Val281Leu) c.920-921insT (c.923dup) c.952C>T (p.Gln318X) c.1066C>T (p.Arg356Trp)	19.2	HC: 20–5 mg (0800–2300 h)	–	0.07
2	37	F	SW	del/conv	del/conv	37.6	HC: 25–10 mg (0900–1700 h)	0.1 mg	0.08
3	33	M	SW	del/conv	c.290-13A/C>G	29.9	HC: 15–5–7.5 mg (9.00 am – 0.30 pm – 11.30 pm) HC: 17.5–10 mg (0730–1830 h)	0.2 mg	0.51
4	52	M	SV	c.515T>A (p.Ile172Asn)	del/conv	29.6	HC: 10–10 mg (0800–2300 h)	–	0.83
5	45	M	SV	c.290-13A/C>G	del/conv	26.2	HC: 6–4–4 mg (0800–1400–2300 h)	0.0625 mg	0.14
6	28	F	SW	del/conv	c.1VS2-13A/C>G	25.7	HC: 6–6–6 mg (0630–1600–2200 h)	0.0625 mg	0.02
7	27	F	SW	c.290-13A/C>G	c.329-336del (p.Gly110fs)	23.4	HC: 17.5–15 mg/HC: 15–15 mg (every other day, 0630–1730 h)	0.1 mg	0.18
8	42	F	SW	c.290-13A/C>G	c.952C>T (p.Gln318X)	29.5	HC: 10–15 mg (0600–0900 h), DXM: 0.25 mg (ante noctem)	0.150 mg	0.17
9	28	F	SW	c.1066C>T (p.Arg356Trp)	c.1066C>T (p.Arg356Trp)	24.5	HC: 10–10 mg (0830–2230 h)	0.0625–0.125 mg (every other day)	0.03
10	28	M	SW	del/conv	del/conv	23.6	HC: 10–15 mg (0600–0900 h), DXM: 0.25 mg (ante noctem)	0.4 mg	0.08
11	48	M	SV	del/conv	c.515T>A (p.Ile172Asn)	24.4	HC: 15–5 mg (0830–2330 h)	–	0.50
12	32	F	SW	c.707T>A (p.Ile236Asn), c.710T>A (p.Val237Glu), c.716T>A (p.Met239Lys)	del/conv	29.5	DXM: 0.1–0.1 mg (0730–2300 h)	0.3 mg	0.44

P, patient number; F, female; M, male; del, deletion; conv, conversion; HC, hydrocortisone; DXM, dexamethasone.

^aSW, classic salt wasting CAH; SV, classic simple virilizing CAH.^bNucleotides are numbered according to the HGVS guidelines using Genbank entry NM_000500.5 as reference sequence, where the A of the first ATG is 1. The variable leucine stretch in exon 1 was counted as 4, not 5 leucines to comply with the widely accepted numbering used in the literature.^cMineralocorticoid medication (9- α -fluorohydrocortisone acetate) was taken in one to two doses.^dSalivary levels of androstenedione are mean levels from six samples (see Materials and methods).

~600 pmol/l) clamp. Glucose tolerance was measured using an oral 75-g glucose load. Blood glucose and insulin samples were collected at 30, 60, 90, and 120 min after the test load. Glucose tolerance was evaluated using the criteria of WHO (21). Body fat distribution was assessed by two different methods, magnetic resonance imaging (MRI) and DEXA scanning. Total body DEXA scanning was performed using a Hologic QDR 4500 densitometer (Hologic, Bedford, MA, USA) to determine total bone mineral content (BMC, g), total areal bone mineral density (g/cm^2), fat mass, and lean mass. Twenty-four-hour ambulatory blood pressure measurement was performed using the ambulatory SpaceLabs model 90207 blood pressure monitoring system. Mean salivary androstenedione levels were calculated from six samples: three samples after placebo treatment and three samples after pioglitazone treatment (same time points as after placebo treatment). Undertreatment was defined as the presence of a mean level of salivary androstenedione above the upper reference morning (0800 h) level, i.e. more than 0.63 nmol/l in males and 1.1 nmol/l in females. Overtreatment was defined as the presence of a mean level of salivary androstenedione below the lower reference morning (0800 h) level, i.e. lower than 0.14 nmol/l in males and 0.16 nmol/l in females.

In control subjects insulin sensitivity was measured using the same euglycemic hyperinsulinemic clamp procedure as in CAH patients.

Procedures

Euglycemic clamp Experiments were performed in the morning after an overnight (10 h) fast. Two i.v. cannulae were inserted. One positioned retrogradely into a dorsal vein of the hand that was placed in a Plexiglas box, ventilated with heated air, for sampling of arterialized venous blood (22). The second cannula was inserted in an antecubital vein of the contralateral arm for infusion of insulin and glucose. Insulin (Actrapid, NovoNordisk, Bagsvaerd, Denmark; diluted in NaCl of 0.9% to a concentration of 1 U/ml, with the addition of 2 ml whole blood per 50 ml) was infused at a rate of 60 mU/min per m^2 body surface area (360 pmol/min per m^2). Arterialized venous plasma glucose was measured in duplicate at 5-min intervals by the glucose oxidation method (Beckman Glucose Analyser II, Beckman, Fullerton, CA, USA). Plasma glucose was clamped at the fasting level by a variable infusion of glucose 20% solution. Plasma insulin was measured in all samples with an in-house RIA at the start of the clamp procedure and after 90 and 120 min (23). Whole body glucose disposal was determined by glucose infusion rate (GIR) as: (mean glucose infusion 90–120 min (mg/min)/weight (kg)) $\times$ (1000/180 g/mol) $\mu\text{mol}/\text{min}$ per kg.

Magnetic resonance imaging and spectroscopy Liver fat content and abdominal fat distribution were determined by proton magnetic resonance spectroscopy (MRS) and imaging (MRI) on a clinical 3T whole body MR system (Siemens Magnetom Tim Trio, Erlangen, Germany). All measurements were carried out during breath-holding for 15 s. Liver fat content was determined by single-voxel proton MR spectra with STEAM localization (echo time 20 ms; repetition time 3 s). Hepatic fat percentage was calculated without correction for differences in relaxation times by dividing the lipid methylene signal intensity at 1.3 ppm by the sum of the methylene lipid and water signal intensities and multiplying the result by 100. Abdominal fat distribution was derived from 16 T1-weighted FLASH-2D axial MR images from a region extending from 4 cm above to 4 cm below the fourth and fifth lumbar interspaces. Subcutaneous and visceral fat volumes were determined based on signal intensity.

Statistical analysis

For calculation and statistical analyses, the SPSS personal computer software package was used (SPSS Inc., Chicago, IL, USA) and $P < 0.05$ was considered statistically significant. Differences between pioglitazone and placebo were statistically tested using Student's *t*-test or the Wilcoxon–Mann–Whitney test as appropriate. According to power calculations, detecting a 20% change in insulin sensitivity with a power of 80% at a significance level of 0.05 would require 11 subjects completing the study.

Results

A total of 12 CAH patients completed the study, seven females and five males. All patients had a biochemically and genetically proven CAH and were treated with stable corticosteroid doses for at least 3 months. Eight patients had the classic salt wasting type of CAH, three patients had the classic simple virilizing type of CAH and one patient had a nonclassical type of CAH. Evaluation of mean salivary androstenedione levels showed that six patients were adequately treated, five patients were overtreated and one patient was undertreated (data shown in Table 2).

Insulin sensitivity

CAH patients were insulin resistant compared with the control group using the euglycemic clamp procedure ($\text{GIR } 28.5 \pm 11.6$ vs $46.2 \pm 23.4 \mu\text{mol}/\text{kg}$ per min, CAH patients versus controls respectively, $P = 0.04$, Fig. 1). Similarly, the insulin sensitivity index in CAH patients was significantly lower compared with control subjects (0.35 ± 0.16 vs $0.56 \pm 0.30 \mu\text{mol}/\text{kg}$ per min (mU/l), $P = 0.03$).

Figure 1 Glucose infusion rate (GIR, $\mu\text{mol/kg per min}$) in control subjects, CAH patients during placebo treatment and CAH patients during pioglitazone treatment, measured during a euglycemic clamp procedure. Mean values for each cluster are shown by the different boxes.

During treatment with pioglitazone, GIR significantly increased compared with placebo treatment (38.9 ± 11.0 vs 28.5 ± 11.6 $\mu\text{mol/kg per min}$, $P=0.000$, Fig. 1). Similarly, insulin sensitivity index improved during pioglitazone treatment compared with placebo (0.53 ± 0.16 vs 0.35 ± 0.16 $\mu\text{mol/kg per min}$ (mU/l, $P<0.001$) (24). A nonsignificant decrease in homeostasis model assessment (HOMA)-estimated insulin resistance (HOMA-IR) was observed after the use of pioglitazone (1.97 ± 1.40 vs 1.80 ± 0.99 , $P=0.34$) (25). Plasma glucose and insulin levels during the clamps were similar during both treatments and in both groups.

Oral glucose tolerance test (OGTT) showed no significant differences in glucose levels after the use of pioglitazone compared to placebo. Insulin levels were lower, although nonsignificantly, after the use of pioglitazone. The insulin_{AUC} (mU/l per h) for the whole test period (0–120 min) was significantly lower after the use of pioglitazone compared with placebo (81 ± 33 vs 111 ± 69 mU/l per h, $P<0.05$), with no significant difference in glucose_{AUC} (mmol/l per h) for the whole test period (13.8 ± 2.5 vs 13.1 ± 3.1 mU/l per h, $P=0.31$).

Body fat distribution

The MRI–MRS data showed no statistically significant changes in subcutaneous fat, visceral fat, the visceral–

Table 3 Magnetic resonance imaging–magnetic resonance spectroscopy measurements of fat distribution, during placebo or pioglitazone in congenital adrenal hyperplasia patients.

	Placebo	Pioglitazone	P value
Subcutaneous fat (l)	1.98 ± 0.83	2.00 ± 0.75	0.76
Visceral fat (l)	0.58 ± 0.30	0.58 ± 0.25	0.93
Visceral:subcutaneous fat ratio	0.3 ± 0.2	0.3 ± 0.2	0.45
% Liver fat	4.2 ± 9.0	3.6 ± 5.6	0.65

subcutaneous fat ratio, and the percentage of liver fat after the use of pioglitazone compared to placebo (Table 3). Most patients had relatively low amounts of liver fat. The only patients with a high liver fat content during placebo (32.7%) showed a strong decrease in the percentage of liver fat after the use of pioglitazone (19.3%). Other patients did not show a decrease in the percentage of liver fat after the use of pioglitazone.

DEXA scanning showed no relevant changes in total body BMC, total lean mass, total fat mass, lean and fat mass in trunk, arms and legs, and the distribution of the fat mass after use of placebo or pioglitazone compared to baseline data.

Blood pressure profiles

Ambulatory 24-h blood pressure was significantly lower during pioglitazone compared with placebo treatment (systolic: 124.0 ± 13.6 vs 127.0 ± 14.9 mmHg, $P<0.001$, diastolic: 72.8 ± 11.5 vs 77.4 ± 12.6 mmHg, $P<0.001$, Fig. 2 and Table 4).

Clinical characteristics, lipid profile, and steroid profile

Clinical characteristics, like weight, BMI, waist, hip, and waist/hip ratio were not significantly different after the use of placebo or pioglitazone. No significant changes in lipid profile and levels of steroids, ACTH, and renin were observed after the use of pioglitazone compared to placebo.

Discussion

The main findings of the present study are that CAH patients who are chronically treated with glucocorticoids are insulin resistant as compared with body mass and age-matched normal subjects and that treatment with pioglitazone improves insulin sensitivity in this group of patients.

The finding that CAH is associated with insulin resistance is in line with earlier studies. These prior studies suggest that both overproduction of androgens and treatment with (supraphysiological doses of) glucocorticoids can induce insulin resistance (5–11, 26, 27).

In our study, we document a significantly lower insulin sensitivity index and GIR in adult CAH patients who were chronically treated with glucocorticoids. The observed insulin resistance may well be the result of treatment with glucocorticoids. Glucocorticoids are known to induce insulin resistance, although the mechanisms involved are incompletely understood (28). Supraphysiological doses of glucocorticoids also increase lipolysis and plasma free fatty acids (FFA), which may lead to insulin resistance in muscles (16, 29).

Insulin resistance is known to be a risk factor for the development of cardiovascular disease and to precede

Figure 2 Twenty-four-hour ambulatory blood pressure during pioglitazone (open squares) or placebo treatment (closed squares) in CAH patients.

the development of type 2 diabetes (30, 31). Therefore, CAH patients might be at risk to develop type 2 diabetes or cardiovascular disease later in life. Because CAH patients need to continue glucocorticoid treatment to suppress overproduction of androgens and as substitution of cortisol, we reasoned that an intervention with insulin-sensitizing drugs, like TZDs, might have a favorable effect on insulin sensitivity in CAH patients and as a result improve their cardiovascular risk profile (15).

We observed a significant improvement of insulin sensitivity (measured both by clamp and by insulin response during OGTT) in CAH patients treated with pioglitazone, although not to the level of the healthy control group. Pioglitazone is known for its ability to improve insulin sensitivity in several conditions associated with insulin resistance. It has been hypothesized that TZDs have an insulin-sensitizing effect because of alterations in adipokine release, which modulates

insulin sensitivity outside adipose tissue (15). TZDs also promote the uptake and the storage of fatty acids in adipose tissue, which does lead to an increase in adipose tissue mass, but in this way TZDs spare other insulin-sensitive tissues as the liver and the skeletal muscle from the harmful metabolic effect of high concentrations of FFA (15).

During pioglitazone treatment, ambulatory 24-h systolic and diastolic blood pressures decreased significantly. It has been noticed before that TZDs can improve blood pressure profiles, although the exact mechanism is not yet known. Insulin resistance is known to be a risk factor for the development of hypertension, so one may hypothesize that improvement of insulin sensitivity may also decrease blood pressure. Insulin resistance contributes to the development of hypertension through several mechanisms like angiotensin II and aldosterone actions, enhanced sympathetic nervous system activity, dyslipidemia, atherosclerosis, left ventricular hypertrophy and changes in renal function and structure, like glomerulosclerosis (32). The anti-hypertensive effects of pioglitazone have been studied in Japanese male patients with type 2 diabetes (33). This study showed a significant decrease in mean blood pressure (109 ± 14 to 101 ± 10 mmHg) after 3 months of treatment with pioglitazone (30 mg/day). Results of the present study are in line with these findings. Anti-hypertensive effects of pioglitazone have also been described in type 2 diabetics with difficult-to-control hypertension, nondipping diabetic patients, and in diet-induced obese rats (34–36).

Although our study shows favorable effects of pioglitazone on insulin sensitivity and blood pressure in adult CAH patients, it has become uncertain whether pioglitazone is a valuable addition to current treatment strategies. Recent studies have questioned the long-term safety of TZDs, especially with respect to long-term cardiovascular outcome. Frequently noticed side effects of TZDs include edema, weight gain, macular edema, and heart failure. Furthermore, TZDs tend to increase low-density lipoprotein cholesterol levels (37). Rosiglitazone may even be associated with a higher risk of myocardial infarction and death due to cardiovascular causes (38), but the RECORD study only confirmed the increased risk of heart failure (39). The PROactive Study showed favorable effects of pioglitazone on reduction of all-cause mortality, nonfatal myocardial infarction, and stroke in type 2 diabetes patients (40). Longer follow-up of treatment with TZDs is needed to evaluate their effects on congestive heart failure and cardiovascular death (41). Treatment with TZDs also decreases bone formation and bone mass, possibly as a result of promoting adipogenesis above osteoblastogenesis (42). This side effect seems particularly relevant in our group of patients, against the background of the already ongoing adverse effect of corticosteroids on the bone. Over this short treatment period, we did not observe any

Table 4 Twenty-four-hour ambulatory blood pressure during pioglitazone or placebo treatment in congenital adrenal hyperplasia patients.

	Placebo	Pioglitazone	P value
24-h SBP	127.0 $\pm$ 14.9	124.0 $\pm$ 13.6	0.000
24-h DBP	77.4 $\pm$ 12.6	72.8 $\pm$ 11.5	0.000
24-h HR	77.1 $\pm$ 13.9	75.1 $\pm$ 13.5	0.007
24-h MAP	93.4 $\pm$ 12.5	89.4 $\pm$ 11.1	0.000
Daytime SBP	129.9 $\pm$ 13.4	127.7 $\pm$ 12.1	0.017
Daytime DBP	79.9 $\pm$ 11.5	75.3 $\pm$ 11.0	0.000
Daytime HR	78.9 $\pm$ 12.9	78.1 $\pm$ 12.9	0.396
Daytime MAP	95.9 $\pm$ 11.3	91.9 $\pm$ 10.3	0.000
Nighttime SBP	116.5 $\pm$ 15.0	111.8 $\pm$ 11.9	0.010
Nighttime DBP	68.3 $\pm$ 12.6	62.6 $\pm$ 9.2	0.000
Nighttime HR	70.2 $\pm$ 13.3	66.6 $\pm$ 10.0	0.048
Nighttime MAP	84.3 $\pm$ 12.6	79.5 $\pm$ 9.2	0.000
Nocturnal drop in SBP (% fall)	10.7	12.2	0.341
Nocturnal drop in DBP (% fall)	14.5	16.5	0.390

SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; MAP, mean arterial pressure.

changes in BMC during treatment with pioglitazone. Nevertheless, with the currently available data on adverse effects of TZDs, the routine use of pioglitazone in CAH patients seems not to be indicated in a clinical setting.

Our study has limitations. Inherent to the short 16-week treatment period, we were not able to evaluate long-term effects of pioglitazone in our population. Furthermore, insulin sensitivity was used as a surrogate outcome, but no data on the prevalence of diabetes mellitus or cardiovascular disease are available. Furthermore, our study was probably underpowered to detect changes in fat distribution, fat mass, and liver fat.

In summary, this study shows that adult CAH patients treated with glucocorticoids are insulin resistant. Treatment with pioglitazone significantly improves insulin sensitivity and decreases blood pressure in CAH patients compared with the use of placebo. Despite these findings, there is currently not enough evidence to warrant the routine use of pioglitazone in this group of patients.

Declaration of interest

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

Funding

The study was supported by an unrestricted grant from Eli Lilly Netherlands (H6E-UT-0013).

Acknowledgements

We thank Iivonne van Loosbroek–Wagemans for her assistance in MR data acquisition and part of the MR data processing. We gratefully acknowledge Vincenzo Positano of the CNR Institute of Clinical Physiology, Pisa, Italy, for providing the HIPPO FAT software to us.

References

- White PC & Speiser PW. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Endocrine Reviews* 2000 **21** 245–291.
- Walker BR. Glucocorticoids and cardiovascular disease. *European Journal of Endocrinology* 2007 **157** 545–559.
- Charmandari E & Chrousos GP. Metabolic syndrome manifestations in classic congenital adrenal hyperplasia. Do they predispose to atherosclerotic cardiovascular disease and secondary polycystic ovary syndrome? *Annals of the New York Academy of Sciences* 2006 **1083** 37–53.
- Bergthorsdottir R, Leonsson-Zachrisson M, Odén A & Johansson G. Premature mortality in patients with Addison's disease: a population-based study. *Journal of Clinical Endocrinology and Metabolism* 2006 **91** 4849–4853.
- Speiser PW, Serrat J, New MI & Gertner JM. Insulin insensitivity in adrenal hyperplasia due to nonclassical steroid 21-hydroxylase deficiency. *Journal of Clinical Endocrinology and Metabolism* 1992 **75** 1421–1424.
- Charmandari E, Weise M, Bornstein SR, Eisenhofer G, Keil MF, Chrousos GP & Merke DP. Children with classic congenital adrenal hyperplasia have elevated serum leptin concentrations and insulin resistance: potential clinical implications. *Journal of Clinical Endocrinology and Metabolism* 2002 **87** 2114–2120.
- Paula FJ, Gouceia LM, Paccola GM, Piccinato CE, Moreira AC & Foss MC. Androgen-related effects on peripheral glucose metabolism in women with congenital adrenal hyperplasia. *Hormone and Metabolic Research* 1994 **26** 552–556.
- Saygili F, Oge A & Yilmaz C. Hyperinsulinemia and insulin insensitivity in women with nonclassical congenital adrenal hyperplasia due to 21-hydroxylase deficiency: the relationship between serum leptin levels and chronic hyperinsulinemia. *Hormone Research* 2005 **63** 270–274.
- Sartorato P, Zulian E, Benedini S, Mariniello B, Schiavi F, Bilora F, Pozzan G, Greggio N, Pagnan A, Mantero F & Scaroni C. Cardiovascular risk factors and ultrasound evaluation of intima-media thickness at common carotids, carotid bulbs, and femoral and abdominal aorta arteries in patients with classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Journal of Clinical Endocrinology and Metabolism* 2007 **92** 1015–1018.
- Bachelot A, Plu-Bureau G, Thibaud E, Laborde K, Pinto G, Samara D, Nihoul-Fékété C, Kuttann F, Polak M & Touraine P. Long-term outcome of patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Hormone Research* 2007 **67** 268–276.
- Völkl TMK, Simm D, Körner A, Rascher W, Kiess W, Kratzsch J & Dörr HG. Does an altered leptin axis play a role in obesity among children and adolescents with classical congenital adrenal hyperplasia due to 21-hydroxylase deficiency? *European Journal of Endocrinology* 2009 **160** 239–247.
- Cornean RE, Hindmarsh PC & Brook CGD. Obesity in 21-hydroxylase deficient patients. *Archives of Disease in Childhood* 1998 **78** 261–263.
- Völkl TM, Simm D, Beier C & Dörr HG. Obesity among children and adolescents with classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Pediatrics* 2006 **117** e98–e105.
- Stumvoll M & Haring HU. Glitazones: clinical effects and molecular mechanisms. *Annals of Medicine* 2002 **34** 217–224.
- Yki-Järvinen H. Thiazolidinediones. *New England Journal of Medicine* 2004 **351** 1106–1118.
- Willi SM, Kennedy A, Wallace P, Ganaway E, Rogers NL & Garvey WT. Troglitazone antagonizes metabolic effects of glucocorticoids in humans: effect on glucose tolerance, insulin sensitivity, suppression of free fatty acids and leptin. *Diabetes* 2002 **51** 2895–2902.
- Willi SM, Kennedy A, Brant BP, Wallace P, Rogers NL & Garvey MT. Effective use of thiazolidinediones for the treatment of glucocorticoid-induced diabetes. *Diabetes Research and Clinical Practice* 2002 **58** 87–96.
- Ginsberg HN. Treatment for patients with the metabolic syndrome. *American Journal of Cardiology* 2003 **91** 29E–39E.
- Tack CJ, Ong MK, Lutterman JA & Smits P. Insulin-induced vasodilation and endothelial function in obesity/insulin resistance. *Diabetologia* 1998 **41** 569–576.
- Levey AS, Bosch JP, Breyer Lewis J, Greene T, Rogers N & Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Annals of Internal Medicine* 1999 **130** 461–470.
- WHO Expert Committee. Second report on diabetes mellitus. *World Health Organization Technical Report Series* 1980 **646** 1–80.
- Abumrad NN, Rabi D, Diamond MP & Lacy WW. Use of a heated superficial hand vein as an alternative site for the measurement of amino acid concentrations and for the study of glucose and alanine kinetics in man. *Metabolism: Clinical and Experimental* 1981 **30** 936–940.
- Abbink EJ, Walker AJ, van der Sluijs HA, Tack CJ & Smits P. No role of calcium- and ATP-dependent potassium channels in insulin-induced vasodilatation in humans *in vivo*. *Diabetes/Metabolism Research and Reviews* 2002 **18** 143–148.
- Bergman RN, Finegood DT & Ader M. Assessment of insulin sensitivity *in vivo*. *Endocrine Reviews* 1985 **1** 45–86.

- 25 Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF & Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting glucose and insulin concentrations in man. *Diabetologia* 1985 **28** 412–419.
- 26 Bayraktar F, Dereli D, Ozgen AG & Yilmaz C. Plasma homocysteine levels in polycystic ovary syndrome and congenital adrenal hyperplasia. *Endocrine Journal* 2004 **51** 601–608.
- 27 Buffington CK, Givens JR & Kitabchi AE. Opposing actions of dehydroepiandrosterone and testosterone on insulin sensitivity. *In vivo and in vitro* studies of hyperandrogenic females. *Diabetes* 1991 **40** 693–700.
- 28 Reynolds RM & Walker BR. Human insulin resistance: the role of glucocorticoids. *Diabetes, Obesity & Metabolism* 2003 **5** 5–12.
- 29 Asensio C, Muzzin P & Rohner-Jeanrenaud F. Role of glucocorticoids in the physiopathology of excessive fat deposition and insulin resistance. *International Journal of Obesity* 2004 **28** S45–S52.
- 30 Rader DJ. Effect of insulin resistance, dyslipidemia, and intra-abdominal adiposity on the development of cardiovascular disease and diabetes mellitus. *American Journal of Medicine* 2007 **120** S12–S18.
- 31 DeFronzo RA. Insulin resistance: a multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia and atherosclerosis. *Netherlands Journal of Medicine* 1997 **50** 191–197.
- 32 El-Atat FA, Stas SN, McFarlane SI & Sowers JR. The relationship between hyperinsulinemia, hypertension and progressive renal disease. *Journal of the American Society of Nephrology* 2004 **15** 2816–2827.
- 33 Hirose H, Kawai T, Yamamoto Y, Taniyama M, Tomita M, Matsubara K, Okazaki Y, Ishii T, Oguma Y, Takei I & Saruta T. Effects of pioglitazone on metabolic parameters, body fat distribution, and serum adiponectin levels in Japanese male patients with type 2 diabetes. *Metabolism: Clinical and Experimental* 2002 **51** 314–317.
- 34 De Rivas B, Luque M, Martell N, Fernández C & Fernández-Crus A. Pioglitazone decreases ambulatory blood pressure in type 2 diabetics with difficult-to-control hypertension. *Journal of Clinical Hypertension* 2007 **9** 530–537.
- 35 Negro R, Dazzi D, Hassan H & Pezzarossa A. Pioglitazone reduces blood pressure in non-dipping diabetic patients. *Minerva Endocrinologica* 2004 **29** 11–17.
- 36 Dobrian AD, Schriver SD, Khraibi AA & Prewitt RL. Pioglitazone prevents hypertension and reduces oxidative stress in diet-induced obesity. *Hypertension* 2004 **43** 48–56.
- 37 Rizos CV, Elisaf MS, Mikhailidis DP & Liberopoulos EN. How safe is the use of thiazolidinediones in clinical practice? *Expert Opinion on Drug Safety* 2009 **8** 15–32.
- 38 Nissen SE & Wolski K. Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. *New England Journal of Medicine* 2007 **356** 2457–2471.
- 39 Home PD, Pocock SJ, Beck-Nielsen H, Curtis PS, Gomis R, Hunefeldt M, Jones NP, Komajda M & McMurray JJV. Rosiglitazone evaluated for cardiovascular outcomes in oral agent combination therapy for type 2 diabetes (RECORD): a multicentre, randomized, open-label trial. *Lancet* 2009 **373** 2125–2135.
- 40 Dormandy JA, Charbonnel B, Eckland DJA, Erdmann E, Massi-Benedetti M, Moules IK, Skene AM, Tan MH, Lefebvre P, Murray GD, Standl E, Wilcox RG, Wilhelmsen L, Betteridge JK, Birkeland K, Golay A, Heine RJ, Korányi L, Laakso M, Mokán M, Norkus A, Pirags V, Podar T, Scheen A, Scherbaum W, Schernthaner G, Schmitz O, Skrha J, Smith U & Taton J. Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (PROspective pioglitazone Clinical Trial In macroVascular Events): a randomised controlled trial. *Lancet* 2005 **366** 1279–1289.
- 41 Lago RM, Singh PF & Nesto RW. Congestive heart failure and cardiovascular death in patients with prediabetes and type 2 diabetes given thiazolidinediones: a meta-analysis of randomised clinical trials. *Lancet* 2007 **370** 1129–1136.
- 42 Grey A. Skeletal consequences of thiazolidinedione therapy. *Osteoporosis International* 2008 **19** 129–137.

Received 17 August 2009

Accepted 7 September 2009