β-cell adaptation in a mouse model of glucocorticoid-induced metabolic syndrome

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Abstract

Glucocorticoids (GCs) are stress hormones primarily responsible for mobilizing glucose to the circulation. Due to this effect, insulin resistance and glucose intolerance are concerns in patients with endogenous overproduction of GCs and in patients prescribed GC-based therapy. In addition, hypercortisolemic conditions share many characteristics with the metabolic syndrome. This study reports on a thorough characterization, in terms of glucose control and lipid handling, of a mouse model where corticosterone is given via the drinking water. C57BL/6J mice were treated with corticosterone (100 or 25 μg/ml) or vehicle in their drinking water for 5 weeks after which they were subjected to insulin or glucose tolerance tests. GC-treated mice displayed increased food intake, body weight gain, and central fat deposit accumulations. In addition, the GC treatment led to dyslipidemia as well as accumulation of ectopic fat in the liver and skeletal muscle, having a substantial negative effect on insulin sensitivity. Also glucose intolerance and hypertension, both part of the metabolic syndrome, were evident in the GC-treated mice. However, the observed effects of corticosterone were reversed after drug removal. Furthermore, this study reveals insights into β-cell adaptation to the GC-induced insulin resistance. Increased pancreatic islet volume due to cell proliferation, increased insulin secretion capacity, and increased islet chaperone expression were found in GC-treated animals. This model mimics the human metabolic syndrome. It could be a valuable model for studying the complex mechanisms behind the development of the metabolic syndrome and type 2 diabetes, as well as the multifaceted relations between GC excess and disease.

Key Words

- glucocorticoid
- diabetes
- insulin secretion
- obesity
- islet cells

Introduction

Glucocorticoid (GC) hormones, like cortisol, are produced and released from the zona fasciculata of the adrenal gland under the control of a prototypic neuroendocrine feedback system of the hypothalamic–pituitary–adrenal (HPA) axis. GCs are stress hormones and secreted in response to a single stimulator, adrenocorticotropic hormone (ACTH), which is released from the anterior pituitary (Feek et al. 1983). ACTH is itself secreted mainly under control of the hypothalamic peptide corticotropin-releasing hormone (CRH). Secreted GC has a negative influence on both CRH
and ACTH release; hence, the steroid regulates its own release in a negative feedback loop. The CNS is thus the commander-in-chief of GC responses, providing an excellent example of close integration between the nervous and endocrine systems (Vegiopoulos & Herzog 2007). GC steroid hormones exert their function in different target tissues by binding to intracellular receptors, either the GC receptor (GR) or the mineralocorticoid receptor (MR).

The GCs are a class of catabolic hormones that are primarily responsible for modulating carbohydrate metabolism (Wajchenberg et al. 1984). In principle, GCs mobilize glucose to the systemic circulation. In the liver, cortisol induces gluconeogenesis and potentiates the action of other hyperglycemic hormones (e.g. glucagon, catecholamines, and growth hormone) on glycogenolysis, which culminates in the release of glucose from the hepatocytes. Cortisol inhibits uptake and utilization of glucose in the skeletal muscle and adipose tissue by interfering with insulin signaling. The hormone also promotes muscle wasting via reduction of protein synthesis and via degradation of protein and release of amino acids. The effect of cortisol on glycemia is further enhanced through the increased breakdown of triglycerides in the adipose tissue, which provides energy and substrates for gluconeogenesis. The increased rate of protein metabolism leads to increased urinary nitrogen excretion and the induction of urea cycle enzymes (Gelfand et al. 1984, Quan & Walser 1992).

Given these effects, insulin resistance and glucose intolerance are concerns both in patients with Cushing’s syndrome and disease (endogenous overproduction of GCs) and in patients prescribed GC-based therapy for immunomodulatory purposes (Raul Ariza-Andraca et al. 1998, Gulliford et al. 2006). In addition, hypercortisolemic conditions share many characteristics with the metabolic syndrome (Anagnostis et al. 2009): a cluster of abnormalities including hyperglycemia, abdominal obesity, dyslipidemia, and hypertension (Kassi et al. 2011, Nikolopoulou & Kadoglou 2012, Mendizabal et al. 2013). In line with the observed similarities between phenotypes associated with the metabolic syndrome and the pathologies associated with GC excess, patients with the metabolic syndrome display elevated levels of cortisol (Duclos et al. 2005, Misra et al. 2008, Sen et al. 2008, Weigensberg et al. 2008) as do patients with glucose intolerance (Reynolds et al. 2001). However, it should be noted that GC elevations in these conditions are far from as apparent as those seen in Cushing’s patients. In addition, dysregulation of the HPA axis has been noted in patients with the metabolic syndrome (Anagnostis et al. 2009). Individuals with central obesity have been reported to have increased urinary free cortisol (Marin et al. 1992), loss of diurnal cortisol variation (Rosmond et al. 1998), and abnormal HPA suppression in response to higher doses of dexamethasone (Pasquali et al. 2002). Considering the phenotypical similarities between GC excess and the metabolic syndrome, there has been a hypothesis formulated that cortisol plays a role also in the metabolic syndrome (Vogelzangs et al. 2007, Anagnostis et al. 2009, Muhtz et al. 2009, Karatsoreos et al. 2010).

To study the mechanisms behind the adverse effects of long-term treatment with GCs, both rat and mouse models have been used. Implantation of corticosterone pellets subcutaneously into Sprague–Dawley rats in combination with a high-fat diet gave rise to glucose intolerance and elevated triglyceride levels in serum, thus mimicking some aspects of the metabolic syndrome (Shpilberg et al. 2012). However this model was not associated with obesity but rather a decrease in body weight, both when GCs were combined with high-fat diet and when given alone. The same phenomena are observed in Wistar rats treated via i.p. injections of dexamethasone for 5 consecutive days (Rafacho et al. 2008, 2010b, 2011). These findings might reflect GC-induced muscle wasting and stress. It has, however, been shown that repeated exposure to corticosterone does not alter muscle strength in rats (Barel et al. 2010). In this context, the reduction in body weight in rats receiving GCs does not completely mimic the phenotype of the metabolic syndrome seen in patients. In contrast to rats, mice exposed to chronic stress develop hypercortisolemia and obesity (Patterson et al. 2013). A potential mouse model for the metabolic syndrome was recently presented by Karatsoreos et al. (2010), in which mice were given corticosterone dissolved in their drinking water. This model has the benefit of reducing stress from animal handling and injections. Here, we report on a thorough characterization of this model in terms of glucose control and lipid handling. We also provide data on the adaptive response to insulin resistance that occurs in the pancreatic islets.

Materials and methods

Animals and treatment

Experiments were performed on 10-week-old male C57BL/6J mice (Nova, Sollentuna, Sweden) which were allowed to feed ad libitum and housed in 12 h light:12 h darkness cycles. Animals were treated with corticosterone...
(100 or 25 μg/ml) or vehicle (1% ethanol) in their drinking water for 5 consecutive weeks after which they were subjected to insulin or glucose tolerance tests (intraperitoneal insulin tolerance test (IPinsTT) and intraperitoneal glucose tolerance test (IPGTT) respectively) and then killed by exposure to CO2. A subgroup of mice was also followed up in a recovery study, where, after the 5 weeks of treatment, the GCs were discontinued and all mice received vehicle for an additional 3 weeks, giving a total study period of 8 weeks. The study was performed according to the guidelines of the Karolinska Institutet and approved by the local animal ethics committee. Food intake, body weight, and fed and fasting blood glucose were monitored weekly, using a hand-held glucometer (One-Touch Ultra 2; LifeScan, Milpitas, CA, USA), and fasting serum was collected for evaluation of insulin levels with ELISA (Mercodia, Uppsala, Sweden). The last week of treatment, mice received i.p. injections of 50 mg/kg body weight BrdU for 7 days. A group of mice was also subjected to blood pressure measurements. After the 5 weeks of treatment, mice were killed and organs were collected. Pancreatic glands were dissected and either used immediately for islet isolation, for evaluation of total insulin content, or fixed in 4% phosphate-buffered paraformaldehyde, paraffin-embedded, and sectioned for immunohistochemistry. Fat deposits were weighed and liver and M. femoralis were snap-frozen for later sectioning and oil red O staining. Blood was collected for analysis of serum nonesterified fatty acids, cholesterol, and triglycerides (analyzed at Karolinska University Laboratory at Södersjukhuset, Stockholm and Center for Inherited Metabolic Diseases, Karolinska University Hospital, Solna) and C-peptide levels were evaluated using ELISA (Alpco Diagnostics, Salem, NH, USA).

IPinsTT and IPGTT

IPinsTT and IPGTT were performed during the fourth or fifth week of treatment and IPinsTT was also performed during the eighth week for the mice in the recovery sub study. For IPinsTT and IPGTT, mice were fasted 1 or 6 h, respectively, and received i.p. injections of insulin (1 U/kg body weight) or glucose (2 g/kg body weight). Blood glucose was monitored at multiple times 0 to 60 (IPinsTT) or 120 (IPGTT) min after injection. In a short version of IPinsTT, mice received insulin or PBS by injection and were killed after 15 min. Organs were snap–frozen and later subjected to western blot analysis for assessing the levels of phosphorylated AKT1/2/3.

Oil red O staining

Frozen pieces of liver and M. femoralis were embedded in NEG-50 (Thermo Scientific, Waltham, MA, USA). Cryosections (12 μm) were obtained from different parts of the tissues and stained for neutral lipids using oil red O (ORO), as previously described (Hagberg et al. 2010).

Mean arterial pressure measurements

Mice were sedated (2% Isoflurane, Apoteket, Uppsala, Sweden) after 4 weeks of GC or vehicle treatment and catheters were surgically placed in the jugular vein for constant saline infusion (10 ml/h per kg) and in the carotid artery for measuring mean arterial pressure (PowerLab, ADInstruments, Spechbach, Germany). Mean arterial pressure was sampled during a 30 min period after surgery and the resting period.

Immunohistochemistry

Paraffin-embedded pancreatic glands were sectioned, stained for insulin (polyclonal guinea pig anti-insulin; DakoCytomation, Glostrup, Denmark) or BrdU (monoclonal rat anti-BrdU; Accurat Chemicals and Scientific Corporation, Westbury, NY, USA), and analyzed as previously described (Grankvist et al. 2012). For each pancreatic gland, the islet volume and BrdU incorporation were determined from all islets identified in nine sections from different parts of the gland. Islet volumes were estimated with the nucleofector method (Bock et al. 2003) using a computerized setup for stereology (newCAST Software, Visiopharm, Hoersholm, Denmark) and presented as mean islet volume. For BrdU incorporation (as a measure of DNA synthesis), BrdU-positive cells were counted and presented as number of BrdU-positive cells divided by islet volume.

Total insulin content

Splenic parts of pancreatic glands were obtained and wet weighed after killing the animal. Acid ethanol (0.18 M HCl in 95% ethanol) was added, pancreatic glands were homogenized by sonication and insulin was extracted over night at 4 °C. Samples were clarified by centrifugation and used for insulin content evaluation using insulin ELISA (Mercodia).

Islet isolation and glucose-stimulated insulin secretion

Pancreatic glands were excised and islets were isolated by collagenase digestion. Islets used for chaperone expression...
were lysed directly after isolation and subjected to western blot or RT for cDNA synthesis and then RT-qPCR. Islets to be used for glucose-stimulated insulin secretion (GSIS) were allowed to recover overnight in culture medium as previously described (Grankvist et al. 2012). Islets were then subjected to GSIS with 30 min preincubation in calcium 5 (Ca5) buffer (containing 25 mM HEPES, 125 mM NaCl, 5.9 mM KCl, 1.2 mM MgCl₂, 1.26 mM CaCl₂, and 0.1% BSA) with 2 mM glucose at 37 °C followed by 60 min incubation in Ca5 buffer with either 2 or 20 mM glucose at 37 °C. Buffers were collected for insulin analysis with ELISA (Mericodia). Islets were then washed with PBS, sonicated, and lysed and DNA was purified, and proteins were removed with phenol–chloroform extraction. DNA concentrations were measured by spectrophotometry (Picodrop Microliter u.v./Vis Spectrophotometer; Picodrop Ltd., Hinxton, UK) and GSIS data were normalized to islet DNA content.

**Protein extraction, SDS–PAGE, and western blot analysis**

Protein samples from isolated islets or other tissues were prepared for western blot analysis as previously described (Sargsyan et al. 2008). Protein concentrations were determined as described by Lowry et al. (1951). Immunoblot analyses were performed using rabbit primary antibodies against CANX, P4HB, HSPA5 (Abcam, Cambridge, UK), CALR, HSP90B1, PDI4, the phosphorylated and total forms of AKT1/2/3 (Santa Cruz Biotechnology), Danvers, MA, USA), DDIT3, and the phosphorylated and total forms of EIF2K3 and EIF2A (Cell Signaling, Cambridge, UK), CALR, HSP90B1, PDIA4, the phosphorylated and total forms of AKT1/2/3 (Santa Cruz Biotechnology). Immunoreactive bands were detected using enhanced chemiluminescence (GE Healthcare, Fairfield, CT, USA), imaged, and quantified using Molecular Imager ChemiDoc XRS with Quantity One Software v. 4.6.5 (Bio-Rad Laboratories). After imaging, the polyvinylidene difluoride membranes were stained with Coomassie Brilliant Blue (Bio-Rad Laboratories) for total protein normalization.

**Islet total RNA extraction, reverse transcription for cDNA synthesis, and quantitative RT-PCR**

Total RNA was extracted from isolated islets using an Aurum Total RNA Mini kit and reverse transcribed into cDNA with iScript cDNA Synthesis kit (Bio-Rad Laboratories). The gene expression levels of mRNAs were measured by SYBR Green-based quantitative real-time RT-PCR (Thermo Scientific) using mouse-specific primers for Calr, Canx, P4Hb, Pdia4, Hsp90b1, and Hspa5. Actb was used as a housekeeping gene for normalization. The following formula was used for quantification: target amount = 2^−ΔΔCt, where ΔΔCt = (Ct_target gene − Ct_Actb)Sample − (Ct_target gene − Ct_Actb)Control (Livak & Schmittgen 2001). For detailed information about the primers, see Supplementary Table S1, see section on supplementary data given at the end of this article.

**Statistical analysis**

Data are presented as mean ± S.E.M. Student’s t-test, or one-way ANOVA followed by Bonferroni post-hoc test, was used as appropriate to identify differences between groups, using GraphPad Prism 5.0 Software (GraphPad, La Jolla, CA, USA). A value of P<0.05 was considered statistically significant.

**Results**

**Corticosterone exposure induces obesity, dyslipidemia, ectopic steatosis, and hypertension**

Mice receiving corticosterone via their drinking water developed enlarged fat deposits (Fig. 1A and Supplementary Figure 1, see section on supplementary data given at the end of this article). The higher dose (100 μg/ml) led to a net body weight gain (Fig. 1B), which was associated with a higher food intake (Supplementary Figure 1D). In a pattern consistent with obesity, mice treated with corticosterone displayed dyslipidemia as indicated by elevated serum levels of nonesterified fatty acids, cholesterol, and triglycerides (Fig. 1C). Also ectopic fat deposition was evident in the model; mice receiving 100 μg/ml corticosterone via their drinking water showed a significant increase in neutral lipid staining in cryosections of liver and skeletal muscle (M. femoralis) compared with vehicle-treated animals (Fig. 1D). Obesity and dyslipidemia are part of the metabolic syndrome and so is hypertension (Kassi et al. 2011). We therefore investigated mean arterial blood pressure after 4 weeks of GC treatment. Mice treated with 100 μg/ml corticosterone had increased mean arterial pressure compared with vehicle-treated animals (Fig. 1E). In conclusion, administration of corticosterone to mice via the drinking water leads to obesity, dyslipidemia, ectopic fat deposition in the liver and skeletal muscle as well as hypertension, thus well mimicking the phenotype seen in patients with the metabolic syndrome.

**Corticosterone exposure induces glucose intolerance and insulin resistance**

To investigate glucose control in the mice treated with corticosterone, serum insulin and blood glucose levels...
were measured every week in fasted or random-fed mice throughout the study period. Corticosterone given via the drinking water dose- and time-dependently increased fasting serum insulin levels (Fig. 2A) compared with vehicle-treated animals. In addition, serum C-peptide levels measured at the end of the treatment period were increased in mice receiving both 25 and 100 \( \mu \text{g/ml} \) (Supplementary Figure 3, see section on supplementary data given at the end of this article). Body weight gain during treatment in mice receiving vehicle (white circles), 25 \( \mu \text{g/ml} \) corticosterone (black triangles) or 100 \( \mu \text{g/ml} \) corticosterone (black diamonds) \((n=6-37)\) is shown in B. Ectopic fat in liver and M. femoralis (D) and mean arterial pressure (E) are shown after 5 weeks treatment with vehicle (white bars) or 100 \( \mu \text{g/ml} \) corticosterone (black bars) \((n=3-6)\).

Images in (D) are representative pictures of ORO staining in the examined tissues \((20 \times \text{magnification, bars represent } 150 \mu \text{m})\). Data are shown as mean \(\pm \text{S.E.M.}\). A * denotes a statistically significant \((P<0.05)\) effect of corticosterone treatment.

**Figure 1**
Corticosterone induces obesity, dyslipidemia, ectopic steatosis, and hypertension in mice. Fat deposits (A) and serum non-esterified fatty acids (NEFA), cholesterol, and triglycerides (C) are shown after 5 weeks of treatment with vehicle (white bars), 25 \( \mu \text{g/ml} \) corticosterone (striped bars) or 100 \( \mu \text{g/ml} \) corticosterone (black bars) via the drinking water. \((n=5-15)\). Body weight gain during treatment in mice receiving vehicle (white circles), 25 \( \mu \text{g/ml} \) corticosterone (black triangles) or 100 \( \mu \text{g/ml} \) corticosterone (black diamonds) \((n=6-37)\) is shown in B. Ectopic fat in liver and M. femoralis (D) and mean arterial pressure (E) are shown after 5 weeks treatment with vehicle (white bars) or 100 \( \mu \text{g/ml} \) corticosterone (black bars) \((n=3-6)\). Images in (D) are representative pictures of ORO staining in the examined tissues \((20 \times \text{magnification, bars represent } 150 \mu \text{m})\). Data are shown as mean \(\pm \text{S.E.M.}\). A * denotes a statistically significant \((P<0.05)\) effect of corticosterone treatment.

Blood glucose levels were not increased in corticosterone-treated mice, irrespective of the dose (data not shown). To further characterize glucose tolerance in corticosterone-treated mice, an IPinsTT and an IPGTT were performed after 4 and 5 weeks of corticosterone treatment respectively. Insulin only modestly decreased glycemia in mice treated with corticosterone, indicating insulin resistance (Fig. 2C). Insulin resistance was further evident from analysis of insulin-induced phosphorylation of AKT1/2/3 in the heart, liver and skeletal muscle tissues; while insulin caused a twofold to threefold induction of phosphorylated AKT1/2/3 in vehicle-treated animals, the hormone failed to induce AKT1/2/3 phosphorylation in corticosterontreated mice (Fig. 2E and F), further demonstrating insulin resistance.

**Research**

L FRANSSON and others

**β-cell adaptation to GC treatment**

DOI: 10.1530/JOE-13-0189

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Corticosterone exposure in vivo induces pancreatic islet growth and enhances insulin secretion capacity

Increased islet size has previously been reported in animal models of obesity and insulin resistance (Rafacho et al. 2009). We measured pancreatic insulin content, islet volume, and islet cell proliferation in samples of the pancreatic gland taken from either vehicle-treated or corticosterone-treated (100 μg/ml) mice after 5 weeks of treatment. Pancreata from corticosterone-treated mice contained 2.3-fold more insulin compared with those from the vehicle-treated animals (Fig. 3A). Immunohistochemical examination of insulin-stained sections revealed that the average islet volume was approximately threefold increased after corticosterone exposure (Fig. 3B). The increased islet volume could be explained by enhanced islet cell proliferation, as the number of BrdU-positive cells per islet volume was increased by a factor of 2.7 (Fig. 3C). We finally analyzed the insulin secretion capacity in islets isolated from vehicle- or corticosterone-treated mice. While there was no difference in the basal insulin release, islets obtained from corticosterone-treated animals
secreted substantially more insulin in response to 20 mM glucose (Fig. 3D). In conclusion, during corticosterone exposure, islet mass and insulin secretion capacity increase presumably in order to compensate for increased insulin demand due to insulin resistance in the peripheral tissues.

**Corticosterone exposure in vivo increases pancreatic islet expression of endoplasmic reticulum chaperones**

Insulin mRNA is translated to preproinsulin which folds in the endoplasmic reticulum (ER) before being packed into secretory vesicles, a process assisted by ER-resident chaperones (Ellgaard & Helenius 2003). Misfolding of insulin can lead to conditions of ER stress and β-cell failure (Ortsater & Sjoholm 2007, Eizirik & Cnop 2010). We analyzed whether the islet levels of ER chaperones were changed to support the increased secretory demand. Western blot analysis of pancreatic islets from corticosterone-treated mice demonstrated that these islets expressed more of the ER chaperones CALR, PDIA4, and HSP90B1, whereas the levels of HSPA5, CANX, and P4HB were only modestly elevated (Fig. 4A). These observations were supported by an analysis of the mRNA expression levels of the same targets (Fig. 4B). In contrast to the increased chaperone expression, phosphorylation of EIF2K3 and EIF2A was not increased in islets isolated from corticosterone-treated mice (data not shown) and islet preparations from both vehicle- and corticosterone-treated mice contained low levels of DDIT3 (data not shown). From these data, we conclude that the increased demand on insulin secretion from the pancreatic islets, in this model with hyperglycemia and insulin resistance, is followed by an adaptation – not only via an increase in the number of β-cells and thus islet size – but also via augmentation of islet ER chaperone expression.

**Discussion**

This study investigates the effects of exogenously administered corticosterone on glucose homeostasis in a commonly used mouse strain, the C57BL/6J. We compared vehicle-treated mice with mice exposed to a high dose of corticosterone (100 µg/ml) as well as with a smaller cohort of mice exposed to a lower dose of the steroid (25 µg/ml). As, only the mice treated with the higher dose of corticosterone lost glucose control and displayed postprandial hyperglycemia, only the higher dose was used for further comparisons.

**Figure 3**

Corticosterone exposure in vivo induces pancreatic islet growth and enhanced insulin secretion in mice. Pancreatic insulin content (A), mean islet volume (B), and number of BrdU-positive cells per pancreatic islet volume (C) are shown after 5 weeks of treatment of mice with vehicle (white bars) or 100 µg/ml corticosterone (black bars). (n = 4–6). B and C show representative immunohistochemistry images of islets for volume comparisons and BrdU staining respectively (20× magnification, bar represents 150 µm). GSIS in isolated islets (D) with low glucose (2 mM, white bars) and high glucose (20 mM, black bars) concentrations are shown after 5 weeks of treatment of mice with vehicle or 100 µg/ml corticosterone. (n = 5–9). Data are shown as mean ± S.E.M. A * denotes a statistically significant (P < 0.05) effect of corticosterone treatment and a # denotes a statistically significant (P < 0.05) effect of high glucose.
A model mimicking the metabolic syndrome

Most importantly, this model of GC excess faithfully recapitulates the phenotype of central obesity seen in patients with Cushing’s syndrome as well as in patients with the metabolic syndrome (Pasquali et al. 2006). The increase in food intake and body weight during treatment was expected and has also been reported by Karatsoreos et al. (2010) together with decreased general home-cage activity. Such a weight gain is, however, not seen in rat strains treated with GCs (Rafacho et al. 2008, 2010b, 2011, Shpilberg et al. 2012), but it is a commonly reported adverse effect of GC treatment in humans (McDonough et al. 2008). Thus, by supplying corticosterone via the drinking water, without any other interventions to healthy mice, a net body weight gain is induced. GC treatment is known to induce muscle wasting and a decline in bone density (Schacke et al. 2002). Although we have not performed a careful examination of lean body mass, the increased size of adipose tissue makes it evident that weight gain resulting from fat accumulation surpasses presumed loss of muscle and bone mass.

Not only did GC treatment induce central fat deposit accumulation, it also led to dyslipidemia (in part likewise found by Karatsoreos et al. (2010)), which is also a prominent feature of the human metabolic syndrome. In addition to dyslipidemia, deposition of ectopic fat in the liver and skeletal muscle was evident in GC-treated mice. Ectopic fat accumulation is a condition often referred to as having a substantial negative effect on insulin sensitivity, which also is a key factor of type 2 diabetes and the metabolic syndrome (Hagberg et al. 2012, Tchernof & Despres 2013). Insulin resistance was consequently demonstrated in this model, evident both when surveying glucose clearance after insulin injection (IPinsTT) and when further studying phosphorylation of AKT1/2/3, a key player in the insulin response required for the glucose transporter 4 to the plasma membrane (Shepherd 2005). In the heart, liver, and skeletal muscles, the insulin-stimulated phosphorylation of AKT1/2/3 was diminished in mice treated with corticosterone, demonstrating a blunted response to insulin in these tissues. Furthermore, the mice exposed to corticosterone were glucose-intolerant as indicated by the reduced glucose clearance after a glucose challenge (IPGTT) as well as the increase in postprandial glycemia. These increased blood glucose levels were evident in the face of increased fasting serum insulin levels, further corroborating the development of insulin resistance. However, the diabetogenic effects of GC excess

Compared with most animal models used to study GC effects, which either rely on adrenalectomy (Christ-Crain et al. 2008) and/or daily injections of GC (Novelli et al. 1999, Rafacho et al. 2008), or surgically inserted GC pellets (Shpilberg et al. 2012), in this study corticosterone was administered via the drinking water. With this approach, animal handling was kept to a minimum and thus inflicted stress (affecting glycemia) was reduced. With the exception of blood sampling once a week, the animals were left undisturbed for 4 weeks after which their insulin sensitivity and glucose handling were investigated via insulin and glucose tolerance tests respectively. In a separate cohort of mice, we also investigated the effects of GC excess on blood pressure. This model with GCs in the drinking water was introduced by Karatsoreos et al. (2010), but in this study we make an in-depth analysis of the metabolic alterations induced by corticosterone.
are transient. When GC treatment was discontinued there was also a remarkably quick – occurring within 1 week – normalization of both serum insulin levels and postprandial hyperglycemia, similar to what has been observed in dexamethasone-treated rats (Rafacho et al. 2010b).

Hypertension is also one of the criteria for the metabolic syndrome in humans (Kassi et al. 2011). Indeed, mice exposed to corticosterone had higher mean arterial pressure compared with vehicle-treated mice. As GCs act by nonspecific binding on the MR in the kidney, sodium retention and potassium excretion increases (Baid & Nieman 2004). Concomitantly, water retention increases, resulting in augmented plasma volume and thus elevation of blood pressure.

Taken together, these findings – showing body weight gain, increased fat deposits, dyslipidemia, ectopic fat accumulation, glucose intolerance, insulin resistance, and hypertension – present a rodent model mimicking the metabolic syndrome in humans, demonstrating most, if not all, of the criteria for the metabolic syndrome (Kassi et al. 2011). This model for studying the metabolic syndrome or effects of GC excess has the advantage of reduced animal stress due to minimal handling when no surgery or daily injections are needed.

**Effects of corticosterone on pancreatic islet adaptation**

Increased islet size has previously been reported in animal models of obesity and insulin resistance (Gepts et al. 1960, Rafacho et al. 2009, Ahren et al. 2010) and this was also evident in this model with GC excess. Both pancreatic insulin content and islet volume were increased in mice exposed to corticosterone. These results suggest that the insulin resistance and increased blood glucose levels in this model give rise to pancreatic islet β-cell compensation due to the increased demand for insulin. Pancreatic β-cells have a substantial potential to adapt, which includes increases in insulin production and insulin secretion and in the long run also a higher islet mass due to increased cell proliferation (Sachdeva & Stoffers 2009). In line with this, this study detected increased BrdU incorporation in islets from mice treated with corticosterone. This suggests that the larger islet size and pancreatic insulin content is due to an increased β-cell proliferation. Also compensation in terms of increased insulin secretion in islets ex vivo was shown in this study. GSIS was increased in islets from corticosterone-exposed mice, with no difference between vehicle- or corticosterone-treated mice at low glucose. Similar results have previously also been found in islets isolated from rats exposed to dexamethasone (Rafacho et al. 2010a). Related to this, we also found augmented expression of ER chaperones in islets from mice exposed to corticosterone. These results suggest that the ER chaperone expression was increased to meet the elevated secretory demand of insulin from the β-cells (Eizirik & Cnop 2010). Enhanced expression of ER chaperones could be an indication of ongoing ER stress. However, as we could not observe increased phosphorylation of either EIF2K3 or EIF2A, and also detected very low and unchanged levels of DDIT3 (which are all markers of ER stress) in the islets obtained from corticosterone-treated mice, it is unlikely that corticosterone has a direct ER stress-inducing effect on islets.

Taken together, we conclude that the increased demand for insulin secretion from the pancreatic islets, in this model with GC-induced hyperglycemia and insulin resistance, is met by an adaptation not only in the number of islet cells and thus islet size but also in increased insulin secretion and in ER chaperone expression. However, while sufficient to maintain fasting normoglycemia, this upregulation of insulin production is inadequate to compensate for the adverse effects of corticosterone on insulin resistance and postprandial hyperglycemia, and the mice display lost glucose control despite the high serum insulin levels.

**Conclusion**

In conclusion, this study reveals new features of the mouse model first described by Karatsoreos et al. (2010). These findings show that the model animals in many aspects mimic the metabolic syndrome in humans, demonstrating a net increase in body weight, dyslipidemia, ectopic fat deposition, and hypertension that occur in conjunction with insulin resistance and glucose intolerance. This study also investigates the plasticity of the pancreatic islets in this setting of GC excess, showing initial compensatory effects on islets and ER chaperones. Furthermore, it is clear that the negative metabolic effects of corticosterone are rapidly reversible as both serum insulin levels and blood glucose levels were promptly normalized within 1 week after GC removal. This model of the metabolic syndrome and GC excess has the advantage of not inflicting additional stress to the animals, due to reduced handling and injections and lack of surgery during the study period. This model may preferably be used in efforts to investigate the complex mechanisms behind development of the metabolic syndrome and type 2 diabetes as well as the multifaceted relations between GC excess and disease.
Supplementary data

This is linked to the online version of the paper at http://dx.doi.org/10.1530/JOE-13-0189.

Declaration of interest

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

Funding

This study was supported by grants from the Diabetes Research and Wellness Foundation, Swedish Diabetes Foundation (Diabetesfonden) and the Tore Nilsson Foundation. H Ortsäter is funded by the Swedish Society for Medical Research. L Fransson is partly funded by KID (Karolinska Institutet, Faculty funds for partial funding of doctoral students).

Author contribution statement

L Fransson, Å Sjöholm, and H Ortsäter conceived and designed the experiments. L Fransson, S Fränzen, V Rosengren, P Wolbert, and H Ortsäter performed the experiments. L Fransson, S Fränzen, P Wolbert, and H Ortsäter analyzed the data. L Fransson and H Ortsäter wrote the paper and S Fränzen and Å Sjöholm edited the paper. All authors have read and approved the final version.

Acknowledgements

The authors thank the personnel at the research center and the animal facility of Södersjukhuset for animal care. We also thank Dr Annika Mehlum and Dr Annelie Falkevall for advice on ORO-staining, Dr Mohamed Eweida and Dr Karatsoreos IN for advice on DNA extraction, and Dr Thomas Nyström for valuable comments on the manuscript.

References


Sen Y, Aygun D, Yilmaz E & Ayar A 2008 Children and adolescents with obesity and the metabolic syndrome have high circulating cortisol levels. Neuroendocrinology Letters 29 141–145.


Received in final form 30 August 2013
Accepted 17 September 2013
Accepted Preprint published online 17 September 2013