Exogenous Glucocorticoids and a High-Fat Diet Cause Severe Hyperglycemia and Hyperinsulinemia and Limit Islet Glucose Responsiveness in Young Male Sprague-Dawley Rats

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Corticosterone (CORT) and other glucocorticoids cause peripheral insulin resistance and compensatory increases in β-cell mass. A prolonged high-fat diet (HFD) induces insulin resistance and impairs β-cell insulin secretion. This study examined islet adaptive capacity in rats treated with CORT and a HFD. Male Sprague-Dawley rats (age ~6 weeks) were given exogenous CORT (400 mg/rat) or wax (placebo) implants and placed on a HFD (60% calories from fat) or standard diet (SD) for 2 weeks (N = 10 per group). CORT-HFD rats developed fasting hyperglycemia (>11 mM) and hyperinsulinemia (~5-fold higher than controls) and were 15-fold more insulin resistant than placebo-SD rats by the end of ~2 weeks (Homeostatic Model Assessment for Insulin Resistance [HOMA-IR] levels, 15.08 ± 1.64 vs 1.0 ± 0.12, P < .05). Pancreatic β-cell function, as measured by HOMA-β, was lower in the CORT-HFD group as compared to the CORT-SD group (1.64 ± 0.22 vs 3.72 ± 0.64, P < .001) as well as acute insulin response (0.25 ± 0.22 vs 1.68 ± 0.41, P < .05). Moreover, β- and α-cell mass were 2.6- and 1.6-fold higher, respectively, in CORT-HFD animals compared to controls (both P < .05). CORT treatment increased p-protein kinase C-α content in SD but not HFD-fed rats, suggesting that a HFD may lower insulin secretory capacity via impaired glucose sensing. Isolated islets from CORT-HFD animals secreted more insulin in both low and high glucose conditions; however, total insulin content was relatively depleted after glucose challenge. Thus, CORT and HFD, synergistically not independently, act to promote severe insulin resistance, which overwhelms islet adaptive capacity, thereby resulting in overt hyperglycemia. (Endocrinology 154: 3197–3208, 2013)
paired (12, 13) insulin secretion depending on the duration of exposure and experimental models used (13–16).

Short-term GC treatment (1–6 h) to isolated islets from lean rodents has been shown to blunt the first-phase and delay the second-phase insulin response thereby reducing the islet insulin response to glucose challenge (12, 14). However, long-term GC exposure (~2 wk of dexamethasone treatment in rats) is associated with increased insulin release with glucose challenge in vitro (17). Together, these results display the vast conflicting observations with respect to GC exposure and islet function.

In addition to peripheral insulin resistance, GC therapy induces elevations in free fatty acids (FFAs) along with increased food consumption and the preference for energy-dense foods (4). Elevations in lipids have long been known to induce β-cell dysfunction in vivo and in vitro—a phenomenon known as lipotoxicity (18) whereby chronically high levels of FFAs lead to a decrease in insulin secretion along with lower insulin gene expression (19). However, in vivo rodent models of high-fat diet (HFD) have not consistently demonstrated impairments of β-cell function (20).

Although GCs and FFAs are often elevated with obesity, prediabetes, and diabetes, rarely have the independent and synergistic effects of these environments been studied in combination on islet function. Recently, we developed a rodent model of GC-induced diabetes in young male Sprague-Dawley rats, showing that a diabetic phenotype can be rapidly induced when animals are exposed to elevations in exogenous corticosterone (CORT), the active GC in rodents, and a HFD (21). The aim of this study was to examine the in vivo and ex vivo islet adaptations that result from this model of diabetes and test the hypothesis that elevated GC exposure in combination with a HFD exceeds the adaptive capacity of the islets to maintain glucose homeostasis. We demonstrate here that short-term (~2 wk) administration of CORT and a HFD results in hyperglycemia despite increases in β-cell mass, largely because of severe whole-body insulin resistance and impaired islet glucose sensitivity.

Materials and Methods

Ethics statement

All experiments were approved by the York University Animal Care Committee in accordance with the Canadian Council for Animal Care guidelines.

Animal characteristics, surgical procedures, and design

Male Sprague-Dawley rats (225–250 g, 6 wk postweaned; Charles River Laboratories, St Constant, Quebec, Canada) were divided into 4 treatment groups (N = 10 per group). Each rat received subcutaneous implantation of either CORT pellets (4 × 100 mg; C2505; Sigma, Oakville, Canada) or wax pellets, as previously described (21), so that 4 groups existed: wax pellet treatment fed a standard diet (placebo-SD; controls); CORT pellet treatment fed a standard chow diet (CORT-SD); wax pellet treatment fed a HFD (placebo-HFD); and CORT pellet treatment fed a high-fat diet (CORT-HFD). All animals were individually housed (lights on 12 h:lights off 12-h cycle) after 1 week of acclimatization and given their respective diets immediately following surgery. CORT was sampled on the evening of the sixth day after pellet implantation via tail nick from each rat at 2000 h and again 12 h later (0800 h). These samples were later analyzed for CORT levels using a RIA kit (MP Biomedical, Solon, Ohio). Blood glucose values were also measured on day 6 (fed state) via a handheld blood glucose meter (Bayer, Contour, New York).

Diet specifications

Standard diet (SD) (Purina Labdiet, 5012, St Louis, Missouri) or a HFD (TD.06414; Harlan Laboratories, Madison, Wisconsin) was provided ad libitum following recovery from pellet implant surgeries. The HFD consisted of 5.1 kcal/g and the SD consisted of 3.4 kcal/g. Total fat content (60% of total calories) of the HFD was composed of 37% saturated fats, 47% monounsaturated fats, and 16% polyunsaturated fats.

Plasma analyses

Animals were administered an oral glucose tolerance test (OGTT; 1.5 g/kg body mass) on day 12 and an insulin tolerance test (ITT) on day 16 by ip insulin injection, as previously described (21). Capillary blood glucose concentration was measured by a handheld glucometer and blood samples from tail nick were collected in EDTA-coated microvette tubes, and plasma was subsequently analyzed for insulin (INSKR020; Crystal Chem, Downers Grove, Illinois) and FFA levels (NEFA kit, HR Series NEFA-HR; Wako Chemicals, Richmond, Virginia). Glucose and insulin area under the curve (AUC) were measured by each individual’s fasting glucose and insulin levels. For technical and experimental reasons, the day of termination ranged from 2 to 5 days after the ITT to allow for subsequent islet isolation studies and trunk blood was collected for hormone analysis. Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) was calculated based on the following equation: Glucose (mM) × Insulin (μ units × L)/22.5. Homeostatic Model Assessment for β-cells (HOMA-β) was calculated based on the following equation: 20 × Insulin (μ units × L)/Glucose (mM) – 3.5, according to Kiraly et al (22).

Assessment of islet morphology

Immunohistochemistry

The entire pancreas was extracted upon euthanization and immersed in 10% buffered Formalin or 4% paraformaldehyde (Sigma) for up to 48 hours. Tissues were washed in 70% ethanol, embedded in paraffin, and later serial sectioned onto slides. A random selection of slides from each group was dehydrated in xylene, dehydrated in ethanol, and washed in PBS. Antigen unmasking/epitope retrieval was performed by high pressureized heat in a 10 mM solution of sodium citrate buffer, pH 6.0. Slides
were incubated in a protein blocking solution (Signet) and then probed for insulin (1:500, guinea pig; Dako, Burlington, Ontario, Canada) and glucagon (1:200, rabbit, Cell Signaling, no. 2760, Boston, Massachusetts; New England Biolabs, Whitby, Ontario, Canada) and proliferating cell nuclear antigen (PCNA) (1:4000, mouse; Cell Signaling no. 2586). After an overnight incubation at 4°C, slides were immunostained with a secondary antibody of biotinylated goat antiguinea pig (Vector Labs BA1000, Burlington, Ontario, Canada at 1:500 dilutions), antirabbit (Vector Labs BA7000 at 1:500 dilutions), and antimouse (Vector Labs BA9200 at 1:500 dilutions) for insulin, glucagon, and PCNA, respectively. A labeling reagent (Ultrastreptavidin horseradish peroxidase, Convance Signet SIG-32242-95, Princeton, New Jersey) and diaminobenzadine (Sigma) were applied to slides until brown staining was visible. Slides were counterstained in hematoxylin (Sigma, Canada). Representative slides of insulin and glucagon were used for measurements of β- and α-cell mass, islet areas, β-cell number, and β- and α-cell size. Terminal transferase-mediated dUTP-digoxigenin nick end-labeling (TUNEL) staining protocols were performed by the University Health Network group at Toronto General Hospital.

Quantification of β-cell mass, islet area, β-cell size, and number of β-cells per islet

Estimated β-cell mass per animal was measured as the product of the total cross-sectional area of insulin-positive stained β-cells divided by total tissue area and multiplied by mass of the isolated pancreas (mg) at euthanization. On average, 1000 to 1500 islets were analyzed per group at 20 × magnification. Tissue areas were objectively quantified, using a positive pixel count algorithm, representing the insulin-positive areas with slide scanner software (Aperio Scanscope CS, Vista, California). The sum of the positive and strong positive pixel areas for each pancreas section was chosen as the best representation of positive insulin stained areas.

All islets were individually circled around the positively insulin-stained cells and were regarded as the total insulin-positive stained areas using a positive pixel count algorithm as previously described. The number of islets per squared millimeter was determined by adding the number of counted islets per slide and then dividing by the total tissue stained area. Islet areas (μm²) were found by adding together the total islet areas and dividing by the total number of islets per slide. To quantify the size of the individual β-cells per islet area, all distinct nuclei surrounded by insulin-positive stained areas were counted as a β-cell within each islet. Then the insulin-stained area on each slide (sum of β-cell area, μm²) was divided by the total number of β-cells counted on each slide to determine β-cell size. This analysis also allowed calculation of the number of β-cells per average islet by taking total islet areas and dividing by previously determined β-cell size. All of these methods were modified protocols from previously published reports by Kiraly et al (22) and Bates et al (23). Measurements of PCNA quantification, α-cell mass, area, size, and number of α-cell were quantified in a manner similar to insulin-positive staining.

Islet isolations and Western analysis

Another set of identically treated animals (5–6 per group) were killed 18 to 21 days after the start of treatment to examine ex vivo islet function and a similar protocol was used with modifications (24, 25). Collagenase solution (20 mL; 4.6 mg per 10 mL of RPMI 1640 without glucose and glutathione [Wisent], 10 mM HEPES, 1% penicillin-streptomycin, glucose [50% dextrose]) was used to digest the pancreas for 20 minutes in a 37°C water bath with periodic agitation. RPMI buffer with 7% fetal bovine serum (FBS) was added to the digested pancreas and then shaken vigorously. Vials were centrifuged for 4 minutes at 1600g at 4°C. The supernatant was discarded and RPMI with 7% FBS was added and gently shaken to dissolve the pellet. The new mixture was filtered through a mesh fabric (500 μm² pore size) into a new conical tube. This new mixture was centrifuged for 4 minutes 1200g at 4°C. The remaining pellet was suspended in Histopaque-1077 (H8889, Sigma, Canada) and topped off with a layer of RPMI without FBS. The mixture was centrifuged 25 minutes at 2700g at 4°C. The mixture was poured into a new conical tube and Kreb’s buffer (125 mM NaCl, 4.7 mM KCl, 1.2 mM, 5 mM NaHCO₃, 2.5 mM CaCl₂, 2.4 mM MgSO₄, 10 mM HEPES, 0.5% BSA, pH 7.4). The mixture was centrifuged 4 minutes at 1400g at 4°C. The remaining pellet was dissolved in Kreb’s buffer and islets were selected and cultured in filtered RPMI buffer overnight (24 h) at 37°C, 5% CO₂ unless islets were used for Western analysis at which point they were collected and stored at −80°C until further analysis. Fifty micrograms of protein were run on SDS-PAGE and proteins were transferred to a polyvinylidifluoride membrane and blocked in 10% powered milk and Tris-buffered saline with Tween 20 for 2 hours. Primary antibodies were protein kinase C-α (PKC; S578, Cell Signaling at 1:500 dilution), phosphorylated-PKC-α (sc-12356; Santa Cruz at 1:1000 dilution), 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1, ab39364; Abcam at 1:500 dilution) and β-actin (ab6276; Abcam at 1:20 000 dilution) were probed overnight at 4°C until the following morning when secondary antibodies were added to membranes of either biotinylated antimouse or antirabbit (at 1:10 000 dilution) were incubated for 1 hour at room temperature and then washed and imaged. Membranes that were probed with p-PKCα antibody were then stripped, blocked, and reprobed with PKCα antibody overnight and incubated in secondary antibodies for 1 hour, washed, and then imaged. Images were detected on a Kodak In vivo FX Pro imager and molecular imaging software (Carestream Image MI SE, version 5.0.2.3.0, Rochester, New York) was used to quantify protein content.

Glucose-stimulated insulin secretion (GSIS) and insulin content measurements

Islets were separated into a 12-well culture plate (6–10 islets/well in 3 batches) and given a preincubation period for 30 minutes in Kreb’s buffer + 0.1 mM glucose + 0.1% BSA dissolved in Kreb’s buffer. Media were flushed and islets were given fresh Kreb’s buffer with 2.8 mM glucose + 0.1% BSA for 1 hour at 37°C, 5% CO₂. Media were changed to Kreb’s buffer with 16.7 mM glucose + 0.1% BSA for 1 hour at 37°C, 5% CO₂. Immediately following each incubation period, media collected were spun at 5000 rpm at 4°C for 10 minutes and frozen at −20°C for further analysis. Islets were placed in 1 mL cold lysis buffer (acid ethanol solution), sonicated (15 s), and centrifuged at 14 500 rpm at 4°C for 10 minutes. Supernatant was collected and stored at −20°C until further analysis of insulin content. Insulin was measured using an RIA kit (Millipore, Billerica, Massachusetts).
 Statistical analyses
 All data are represented as means ± SE, with a criterion of $P < .05$ and $P < .001$, and were assessed using one-way and two-way ANOVAs as means of statistical significance except for determination of pellet mass differences between CORT treatment groups and acute insulin response (AIR) between placebo-SD and CORT-HFD where an unpaired Student’s $t$ test was used. Individual differences were evaluated using Tukey HSD post-hoc test (Statistica 6.0 software), with adjustments made for multiple comparisons. In each figure different letters denote statistical significance between groups. All fold differences are expressed as relative to the placebo-SD group.

Results

Exogenous CORT increases plasma CORT and abolishes diurnal rhythm
 After 2 weeks of pellet implantation (at the time of euthanization), there were no significant differences found in the pellet mass excised from CORT-SD and CORT-HFD animals (Table 1), thereby suggesting that CORT absorption was not influenced by dietary treatment. CORT levels were higher at peak (2000 h) than at trough (0800 h) in placebo-treated animals 2 weeks after pellet implantation (Table 1). In contrast, CORT-treated animals had elevated basal CORT values that were indistinguishable from peak concentrations. Left and right adrenals were ~50% smaller in CORT-treated animals compared to the two placebo groups, confirming that the exogenous CORT promoted feedback inhibition of ACTH release ($P < .05$, Table 1).

CORT treatment decreases lean mass, increases adipose tissue mass, and promotes hyperglycemia
 All groups were similar in body mass prior to treatment (day 0). Six days following CORT implantations, CORT-treated animals were smaller in mass compared to placebo-treated animals ($P < .05$, Table 2). Relative food intake was increased in CORT-HFD animals compared to placebo-HFD at day 6 ($P < .05$, Table 2). HFD resulted in greater epididymal fat pad mass compared to placebo-SD animals, with the greatest increase observed in the CORT-HFD group ($P < .05$, Table 2). Both CORT-treated groups had elevated fed blood glucose levels relative to the placebo groups on day 6 (main effect of treatment, $P < .05$). Nonesterified fatty acids were elevated in all treatment groups compared to placebo-SD, but were highest in the CORT-HFD group ($P < .05$, Table 2).

Severe glucose intolerance and hyperinsulinemia were induced with CORT-HFD treatment
 Fasting blood glucose levels were the highest in CORT-HFD animals, whereas all other treatment groups had similar fasting glucose levels ($P < .05$, Figure 1A). Glucose AUC during an OGTT was also highest in CORT-HFD animals compared to all other groups ($P < .05$; Figure 1A’). Fasting insulin levels were highest in CORT-HFD animals compared to all other treatment groups ($P < .05$; Figure 1B). Insulin AUC was higher in CORT-SD than in the controls and CORT-HFD animals ($P < .05$; Figure 1B’). AIR is thought to be an early defect in the course of diabetes development and this response can be estimated as the change in insulin levels during the first 15 minutes following oral glucose gavage in rats (26). CORT-HFD animals demonstrated lower AIR compared to CORT-SD and placebo-SD and placebo-HFD groups ($P < .05$, Figure 1C). Figure 1D demonstrates the relationship between fasting glycemia and insulin levels in each of the treatment groups, indicating that higher glucose concentrations were associated with increased insulin levels ($r^2 = 0.42$, $P < .05$). This regression is based on all groups plotted on the same graph; independently the linear regressions are as follows: placebo-SD, $r^2 = 0.005$, CORT-SD, $r^2 = 0.003$, placebo-HFD, $r^2 = 0.04$, and CORT-HFD, $r^2 = 0.02$ (not statistically significant, independently). HOMA-IR index indicated that CORT-HFD animals were ~15-fold more insulin resistant than placebo-SD and

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<th>Table 1. Plasma Corticosterone Levels (ng/mL), Adrenal Mass (mg/BM), and Pellet Mass (mg) After 2 Weeks of Treatment</th>
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Abbreviation: BM, body mass. All values are means ± SE.

- $a$ Value shown is the estimated amount of CORT dissolved over 2 weeks.
- $b$ Statistical significance between AM and PM corresponding CORT levels within each treatment group ($P < .05$).
- $c$ Statistical significance from placebo-SD (controls) during corresponding time points that CORT levels were measured ($P < .05$).
- $d$ Statistical significance of corresponding left and right adrenals from controls.
are means compared to placebo-SD controls (Figure 4A; P<.05). CORT treatment resulted in larger islet area with a greater number of smaller β-cells as compared to placebo controls (Figure 4, C, D, and F; P<.001). Islets were also grouped into various size groupings to demonstrate the differences in islet size distributions (Figure 4E). CORT treatment resulted in ~20% fewer islets in the smallest size group (<5000 μm²) and ~15% more islets in the largest size group (15 000+ μm²) compared to placebo-treated animals, regardless of diet.

Plasma glucagon levels were elevated in CORT-HFD animals compared to placebo-SD (P<.05; Figure 5A). In addition, the CORT-HFD group had the largest α-cell mass and size (μm²) compared to all other treatment groups (Figure 5, B and C; P<.05), indicating α-cell hypertrophy.

**CORT treatment increases β-cell secretory capacity**

It has been previously reported that PKCa plays an essential role in the insulin secretory process and its dysfunction may contribute to hyperglycemia in GC-treated rodents (28). Protein content of total PKCa normalized to β-actin was more highly expressed in islets treated with CORT regardless of diet (Figure 6A; P<.05), suggesting that CORT treatment increases total PKCa expression, perhaps as a result of increased β-cell proliferation. Protein content for p-PKCa normalized to β-actin was up-regulated in the islets derived from CORT-SD, but not in CORT-HFD, rats compared to the islets from the placebo-SD rats (Figure 6B; P<.05).

**CORT treatment increases islet 11β-HSD1 expression**

The activity of tissue-specific 11β-HSD1 manipulates the level of active CORT in various organs in the body.
Moreover, this enzyme has been shown to decrease GSIS in pancreatic murine and human islets (30), potentially contributing to diabetes development (31). Therefore, we assessed the protein content of 11β-HSD1 to determine a functional relationship between insulin secretion and active GC regeneration in the islets. The protein content of 11β-HSD1 was up-regulated in the islets from CORT-treated animals compared to placebo-treated groups, regardless of diet (Figure 7; \(P < .05\)).

CORT-HFD isolated islets exhibit enhanced insulin responses to glucose

To determine ex vivo β-cell function, islets were isolated from all treatment groups, stabilized in culture, and then subjected to low and high concentrations of glucose (2.8 and 16.7 mM). CORT-HFD islets released more insulin than the islets from all other groups in both glucose conditions (Figure 8A; \(P < .05\)), although the fold change in insulin secretion from the low-glucose to the high-glucose media was lowest in the placebo-HFD and CORT-HFD islets (Figure 8A'). Total insulin content was measured after the islets were exposed to high levels of glucose in all treatment groups. CORT-HFD islets expressed the lowest levels of insulin content compared to all other treatment groups (Figure 8B; \(P < .05\)), possibly indicating a greater depletion of insulin in response to high glucose in the media.

Discussion

Exogenous GCs cause insulin resistance and may promote diabetes development (32). A HFD in conjunction with exogenous GCs may increase diabetes risk, although few studies have investigated the amalgamation of these treatments. In this study, we show that the combination of CORT and a HFD results in severe insulin resistance with β-cell proliferation that promotes fasting hyperinsulinemia, but this adaptation is insufficient to maintain glucose homeostasis, particularly in response to an oral glucose challenge. We also show that, at least in the short term, β-cell apoptosis is not increased with CORT and a HFD, but an impairment in β-cell glucose sensing in vivo, likely as a result of chronic hyperglycemia and profound insulin resistance that overwhelms insulin secretory capacity.

Although a number of previous studies have investigated the physiological adaptations to HFD (reviewed in Ref. 20) and exogenous CORT treatment alone (10, 12, 13, 28, 33, 34), we are aware of only one other study that has combined the two to examine potential synergistic effects on islet function and diabetes development (26). In that investigation, dexamethasone (~100 μg kg\(^{-1}\) body weight day\(^{-1}\); 5 d) and a HFD acted synergistically to
induce glucose intolerance by impairing GSIS in vivo, similar to our findings (Figure 1). Moreover, short-term GC treatment (1–6 h) to isolated islets from lean rodents has been shown to blunt the first-phase and delay the second-phase insulin response (12, 14). In contrast to these in vivo and ex vivo findings, previous studies conducted in various rodent models have demonstrated that prolonged GC exposure (equal to or longer than 5 d) increases GSIS (2, 10, 17, 26). Interestingly, and similar to our observations, GCs administered to healthy men cause impairments in several β-cell insulin secretory pathways and has been shown to inhibit the normal insulin response (7). Moreover, first-degree relatives of patients with T2DM who are treated with corticosteroids develop severe insulin resistance and may develop impaired glucose tolerance, depending on their islet function (35). Thus we conclude that in our model there is detriment to β-cell function by placing severe demands on insulin requirements in a fasted state and a blunted capacity to respond to glucose challenge.

As expected, based on our previous study (21), we found that exogenous GCs promote hyperinsulinemia, in both the fasted and the fed state (OGTT) (Figure 1A and Table 2), but that the addition of a HFD markedly increases glucose intolerance and prevents the appropriate insulin response to a glucose challenge (Figure 1, A and B). In a previously mentioned study (26), dexamethasone and a HFD precipitated glucose intolerance in rats by impairing the negative feedback loop between insulin sensitivity and secretion. Similarly, our study demonstrates the rapid onset of overt diabetes that occurs within 1 to 2 weeks and with no evidence of β-cell mass decompensation but with obvious impairments in insulin responsiveness to oral glucose challenge (Figure 1B’). Indeed, CORT-HFD animals demonstrate impaired first-phase insulin response during glucose challenge as measured by the acute insulin response (Figure 1C), suggestive of impaired glucose sensitivity in vivo. On the other hand, it is unlikely 2 weeks of CORT-HFD treatment resulted in complete destruction of β-cell insulin secretion and more likely that the rise in hyperglycemia/hyperinsulinemia was driven by increased peripheral insulin resistance.

Separately, both elevated CORT and a HFD increase peripheral and islet insulin resistance (20, 36), reduce insulin-mediated glucose disposal, and lower muscle glycogen synthesis (37, 38). We found a modest association between fasted glucose and insulin levels ($r^2 = 0.42$, Figure 1D), thereby suggesting that severe hepatic insulin resistance induces fasting hyperglycemia in CORT-HFD animals, as documented previously (39). Indeed, this model...

Figure 2. Measurements of insulin resistance, β-cell function, and insulin sensitivity. HOMA-IR index indicates whole-body insulin resistance (A) and HOMA-β index indicates β-cell function (D). Insulin sensitivity during an ip administered insulin tolerance test. Glucose (mM) levels after insulin injection (C) and glucose AUC (C’). N = 9–10 for each group. In all graphs, bars that do not share the same letters are statistically significant from each other (P < .05). All values are means ± SE. Note that the closed triangles in C are obscured by the closed squares.

Figure 3. Images of islet morphology indicating insulin (A–D), glucagon (E–H), TUNEL (I–L), and PCNA (M–P) positive staining. Arrows indicate apoptotic cells that are TUNEL positive. All images are 20 × magnification.
of short-term CORT treatment induced a 5-fold increase in HOMA-IR compared to controls and the addition of a HFD exacerbated insulin resistance by ~15-fold compared to controls (Figure 2A). Therefore, the combination of the two treatments appears to be the driving force for elevated fasting glucose and insulin levels. To determine if CORT and HFD treatment affect β-cell function, a HOMA-β index was used. CORT-SD treated animals had improved β-cell function compared to all other treatment groups but the addition of HFD decreased β-cell function relative to CORT-SD animals, such that values were similar to what was observed in the placebo-treated groups (Figure 2B). One interpretation is that CORT-HFD treated rats had normal β-cell function, but were in an environment of severe insulin resistance that requires enhanced function. In addition to impairments in hepatic insulin sensitivity and possibly β-cell function, our model demonstrates decreases in skeletal muscle insulin-mediated glucose uptake, as evidenced by poor glucose responsiveness to exogenous insulin challenge (Figure 2, C and C'). Together, these results show that elevations in CORT and HFD induce severe peripheral insulin resistance, reflected by direct and indirect effects on the liver, skeletal muscle, and β-cell function, which represents characteristics similar to those with Cushing’s syndrome or with GC-induced diabetes (2, 35).

A HFD given to rodents has been shown to up-regulate islet mass due to an increase in compensatory β- and ɑ-cell mass (40). However, prolonged elevation in FFAs results in β-cell death (41) and can cause the accumulation of ceramides that impair insulin processing (ie, irregular folding of the insulin peptide) (19) and transcription (42). However, we found no evidence that HFD and CORT caused increased β-cell death, at least in the short term (Figure 3, I–L), but that this treatment significantly increases islet areas through increased β- and ɑ-cell mass (Figure 3, A–H). PCNA staining was significantly up-regulated in the CORT-treated animals, suggestive that pro-

Figure 4. Insulin-positive staining indicates β-cell mass (A), number of islets per pancreas area (mm²) (B), islet area (μm²) (C), number of β-cells per islet (D), percentage of islets per size grouping (μm²) (E), and β-cell size (μm²) (F). N = 6–7 per group. In all graphs, bars that do not share the same letters are statistically significant from each other (graph A–C and E, P < .05, graph D and F, P < .001). *, Statistical significance where P < .05. #, Statistical significance where P < .001 (E). All values are means ± SE.
liferation was increased in response to the insulin resistance (Figure 3, M–P). However, despite the fact that there is clear evidence of islet adaptation to CORT-HFD treatment, these islets are still unable to maintain euglycemia, even in the fasted state.

Very few studies have examined the effects of HFD and/or short-term CORT treatment on glucagon secretion. Interestingly, fasting plasma glucagon increases after dexamethasone treatment in insulin-resistant first-degree relatives of patients with T2DM (35). We demonstrate here that circulating plasma glucagon levels are increased with CORT and HFD (Figure 5A), along with increases in α-cell mass (Figure 5 B and C), which likely further exacerbates hyperglycemia. We found that α-cell mass is up-regulated in CORT-HFD-treated rats primarily as a result of increased α-cell hypertrophy, as there was no change found in α-cell number but the treatment induced increased α-cell size. Early stages of diabetes are associated with α-cell dysfunction as glucagon is secreted at abnormally high levels during an oral glucose challenge (43) and α-cell mass increases linearly with a rise in peripheral insulin resistance (44). Although the mechanisms for increased α-cell mass with CORT treatment are unknown,
it may be an additional diabetogenic feature of the combination of exogenous CORT and HFD treatment. There are several crucial elements involved in the insulin secretory system (45), such as PKC, which is an important regulatory enzyme involved in insulin secretion by activating the movement of \(\beta\)-cell insulin granules for exocytosis (46–49). GC administration in rats results in an up-regulation of the PKC activation pathway, induced by increased \(\text{Ca}^{2+}\) release from intracellular stores, thereby leading to increased insulin secretion (28). Similar to previous findings by Rafacho et al (28), we demonstrate here that GCs in combination with a SD increases both total PKC expression and islet p-PKC content (Figure 6). However, unique to our study, we show that the addition of HFD with CORT administration decreases islet p-PKC content compared to CORT-SD animals. As previously described (50), chronic high glucose impairs normal PKC phosphorylation, thought to induce islet desensitization, thereby limiting insulin secretion. Furthermore, a study in the GK rat, which spontaneously develops impaired \(\beta\)-cell function, suggests impairments in PKC activation (51). Thus, we speculate that at least a part of the impaired insulin response in the AIR of CORT-HFD animals may result because of reduced PKC activation, partly due to sustained hyperglycemia, although it should be noted that PKC regulation is not the only potential mechanism involved in insulin secretion and therefore other key regulatory proteins may be at play.

Elevated tissue GC excess, driven by increased levels of the intracellular GC regenerating enzyme 11\(\beta\)-HSD1 in adipose tissue, liver, and skeletal muscle, is implicated in the development of insulin resistance (52). Elevated 11\(\beta\)-HSD1 activity and/or expression is also found in pancreatic islets of obese/diabetic rodents (53) as well as lean mice treated with GCs (11), but the functional consequence of this increase is unclear. It is proposed that the increase in GC reactivation caused by elevated 11\(\beta\)-HSD1 activity suppress insulin secretion (53), thereby promoting diabetes development. Recently, however, the use of a \(\beta\)-cell-specific 11\(\beta\)-HSD1 overexpression model suggests that elevations in this pre-receptor enzyme actually improves insulin release mechanisms and protects against HFD-induced diabetes (54). In this study, we found that CORT treatment increases 11\(\beta\)-HSD1 protein content in islets, independent of diet (Figure 7). These results are supported by previous work, showing that islets cultivated in the inactive GC (11-dehydrocorticosterone) results in a rapid doubling in 11\(\beta\)-HSD1 protein (31) and provides further evidence that elevations in this enzyme may be a compensatory mechanism supporting insulin hypersecretion (11, 54).

Elevated fatty acid exposure (48 h) leads to the reduction of GSIS in vitro (55); however, in vivo GSIS reports are relatively inconsistent and may be due to variations in insulin sensitivity within individual islets (20). Short-term CORT treatment has been shown to enhance insulin release in vivo (28) by up-regulating IRS-2 signaling pathway (56, 57). We exposed isolated islets to low- (2.8 mM) and high-glucose (16.7 mM) concentrations and found
that islets derived from CORT-HFD animals had greater GSIS compared to all other groups (Figure 8A). This may be explained, however, by the greater β-cell mass in the CORT-treated animals. Moreover, CORT-HFD islets that were subjected to high glucose exposure ex vivo demonstrated lower total insulin storage compared to all other groups (Figure 8B) and this was likely due to increased insulin secretion during the glucose challenge. In addition, it should be noted that although CORT-HFD islets have higher insulin secretion in high glucose media, their relative fold increase over that observed in the low glucose media is less than that observed in SD groups (Figure 8A'). These findings on isolated islets suggest that, although CORT-HFD-treated animals are able to secrete high amounts of insulin in response to glucose in vivo, that ex vivo they do suggest some forms of islet desensitization to changes in glucose concentrations.

Conclusions

In summary, short-term exposure to CORT and a HFD play a synergistic role in the development of insulin resistance, β-cell mass compensation, and relative fasting hyperinsulinemia. However, in vivo, insulin responsiveness is blunted following oral glucose load in CORT-HFD-treated animals despite the increase in β-cell mass. These disturbances are associated with increases in circulating glucagon levels and islet 11β-HSD1 protein expression. In contrast, ex vivo, islets derived from CORT-HFD animals exhibit an up-regulated absolute insulin response, although the fold change from low-glucose exposure is lower. Taken together, these results demonstrate that the combination of exogenous CORT treatment in conjunction with a HFD induces severe hyperinsulinemia/hyperglycemia as a result of whole-body insulin resistance, hyperglucagonemia, and an inappropriate responsiveness of β-cells to glucose challenge.

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