CHAPTER 2

Islet-cell Dysfunction Induced by Glucocorticoid Treatment: Potential Role for Altered Sympathovagal Balance?

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ABSTRACT

Aim
Glucocorticoids impair glucose tolerance by inducing insulin resistance. We investigated the dose-dependent effects of glucocorticoid treatment on islet-cell function in healthy males and studied the role of the autonomic nervous system.

Design and Methods
A randomized, placebo-controlled, double-blind, dose-response intervention study was conducted in 32 healthy males (age: 21±2 years; BMI: 21.9±1.7 kg/m²). Participants were allocated to prednisolone 7.5 mg once daily (n=12), prednisolone 30 mg once daily (n=12), or placebo (n=8) for two weeks. Beta-cell function was measured by hyperglycemic clamp with arginine stimulation, glucagon levels were measured following a standardized meal test.

Results
We found that prednisolone treatment dose-dependently reduced C-peptide secretion following arginine stimulation on top of hyperglycemia (ASI-iAUC$_{CP}$): -2.8 (-5.2;0.2) and -3.1 (-8.8;-1.0) nmol L$^{-1}$min$^{-1}$ for prednisolone 7.5 mg and prednisolone 30 mg, respectively (P=0.035 vs. placebo). Fasting glucagon levels increased dose-dependently (vs. placebo; P=0.001), whereas postprandial glucagon levels were only increased by prednisolone 30 mg. Changes in parasympathetic activity related with changes in fasting glucose levels (r=-0.407; P=0.03) and showed a trend towards correlation with fasting glucagon concentrations (r=-0.337; P=0.07). The change in sympathovagal balance was inversely related to ASI-iAUC$_{CP}$ (r=-0.365; P=0.05).

Conclusion
We conclude that in addition to inducing insulin resistance, prednisolone treatment dose-dependently impaired islet-cell function. Altered sympathovagal balance may be related to these effects.

ISRCTN
78149983
Glucocorticoids (GCs) are the cornerstone in the treatment of numerous diseases due to their potent anti-inflammatory and immunosuppressive actions [1]. However, pharmacological GC levels also induce adverse effects on glucose metabolism [1, 2]. In population-based studies, GC therapy was associated with incident diabetes [3]. Classically, the association between GCs and diabetes has been attributed to GC-induced insulin resistance [4].

The extent to which pancreatic islet-cell dysfunction, and particularly beta-cell dysfunction, contributes to the diabetogenic effects of GCs is less well-known. In vitro, GCs were shown to decrease insulin secretion and insulin synthesis [2]. In addition to reducing glucose-stimulated insulin secretion (GSIS), GCs impaired the in vitro effects of nonmetabolizable insulin secretagogues, including arginine and acetylcholine, suggesting that the site of action of GCs is in the end of the insulin secretory process [5]. Transgenic mice with a beta-cell specific overexpression of the glucocorticoid receptor (GR) develop diabetes due to beta-cell failure, in the presence of increased a2 adrenergic activity [6].

In humans, a single high-dose of prednisolone (PRED) was shown to impair both first-phase glucose-stimulated and arginine-stimulated C-peptide secretion during a hyperglycemic clamp [7] and glucose sensitivity of the beta cell during a meal challenge test [8]. (Sub)acute, high-dose GC-exposure (i.e. 2 day-treatment), however, generated seemingly opposing results [9-13], where most of these studies reported increased insulin secretion during a hyperglycemic clamp or intravenous glucose tolerance test. However, as previously indicated, GCs also induce insulin resistance and the observed increment in glucose-stimulated insulin secretion (GSIS) may be secondary to reduced insulin sensitivity. Only one of these studies measured insulin sensitivity with the hyperinsulinemic-euglycemic clamp technique [11], allowing adjustment for prevailing insulin sensitivity. This study reported impaired compensation for reduced insulin sensitivity in several subjects. This importantly illustrates that beta-cell function and insulin secretion rates per se are not synonymous. In order to assess beta-cell function, insulin secretion rates should always be related to prevailing glucose levels or insulin sensitivity. Other evidence for GC-induced beta-cell dysfunction comes from observations in subjects with diabetes, where suppression of insulin/glucose ratio's were reported during treatment with PRED 20 mg daily, particularly in the morning a few hours following ingestion of study medication [14].

In addition to beta-cell function, GCs may also affect pancreatic alpha-cell function. Two studies showed increased glucagon secretion during high-dose GC treatment [15, 16].

However, there are no data available regarding the effects of more prolonged GC treatment, i.e. past the (sub)acute effects, on islet-cell function in humans. Also, the dose-dependency of these
effects is largely unknown. Finally, mechanisms that could contribute to GC-induced effects on islet cell function in humans have, to our knowledge, not been investigated. Given the preclinical data [5, 6], alterations in the autonomic nervous system (ANS) balance could be implicated in GC-induced islet effects. Whereas parasympathetic branches of the ANS are well-known to stimulate insulin secretion via acetylcholine signaling [17], sympathetic fibers decrease insulin release via catecholamine-related pathways [18] and stimulate glucagon release [19]. Also, it is at present unclear whether the incretin hormones, important regulators of postprandial islet-cell function, may be involved in GC-induced islet effects.

Therefore, in the present study, we assessed the dose-dependent effects of GC treatment on islet-cell function in healthy men and measured cardiovascular ANS balance and meal-related incretin responses. To this end, both low- and high-dose PRED, a very commonly prescribed GC compound, were administered for a period of two weeks.

RESEARCH DESIGN AND METHODS

Participants
Thirty-two healthy Caucasian males were recruited by local advertisement. Inclusion criteria included: age 18-35 years, body mass index (BMI) 20.0-25.0 kg/m², good physical health (determined by medical history, physical examination and screening blood tests) and normoglycemia as defined by fasting plasma glucose (FPG) < 5.6 mmol/L and 2-h glucose < 7.8 mmol/L following a 75g oral glucose tolerance test (OGTT), performed at screening visit. Exclusion criteria were the presence of any disease, use of any medication, first-degree relative with type 2 diabetes, smoking, shift work, a history of GC use, excessive sport activities (i.e. > two times/week) and recent changes in weight or physical activity. The study was approved by the ethics committee of the VU University Medical Center (Amsterdam, the Netherlands, FWA00017598) and the study was conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent before participation.

Study Design
The study was a randomized, placebo-controlled, double blind, dose-response intervention study. Following assessment of eligibility and baseline measurements, participants were randomized to receive either PRED 30 mg once daily (n=12), PRED 7.5 mg once daily (n=12) or placebo (PLB) (n=8) treatment for a period of 14 days using block randomization, as carried out by the Department of Experimental Pharmacology of the VU University Medical Center. These dosages of PRED were chosen as typical high-dose (so called induction dosage for initial treatment) and as typical low-dose (so called maintenance dose for prolonged treatment) in clinical practice. An outline of the study design is presented in Supplementary Figure 1A. At baseline and day 13 of treatment,
insulin sensitivity and beta-cell function were measured in a combined euglycemic-hyperglycemic clamp procedure (Supplementary Figure 1B). At baseline and day 14 of treatment, a standardized consecutive meal challenge test was performed and cardiovascular ANS function was measured in the fasted state (Supplementary Figure 1C). All measurements were conducted following a 12-h overnight fast with the subjects in the semi-supine position. Subjects refrained from drinking alcohol for a period of 24h before the study days and did not perform strenuous exercise for a period of 48h before the study days. During all visits, including a follow-up visit at day 7 of treatment, safety and tolerability were assessed. A patient flow diagram is shown in Supplementary Figure 2.

Hyperinsulinemic-Euglycemic Clamp and Hyperglycemic Clamp

After an overnight fast, participants were admitted to the clinical research unit at 7.30 AM. An indwelling cannula was inserted into an antecubital vein for infusion of glucose and insulin. To obtain arterialized venous blood samples, a retrograde cannula was inserted in a contralateral wrist vein and the hand placed in a heated box, maintained at 50°C. A primed, continuous ($40 \text{ mU m}^{-2} \text{min}^{-1}$) insulin infusion (Actrapid; Novo Nordisk, Bagsvaerd, Denmark) was given for 120 min; plasma glucose was kept at 5 mmol/L by a variable infusion of 20% glucose, as described previously [20]. The hyperglycemic clamp was started 60 min after cessation of exogenous insulin infusion. Plasma glucose concentration was then raised to 10 mmol/L by a body weight-adjusted intravenous bolus of 20% glucose and a variable 20% glucose infusion was adjusted to maintain the targeted glucose level. After 80 min hyperglycemia, an intravenous bolus of 5 g arginine (dissolved in 50 mL NaCl) was given over 45 seconds, and the glucose level was maintained at 10 mmol/L for an additional 30 min (Supplementary Figure 1B).

Standardized Consecutive Meal Challenge

After an overnight fast, participants were admitted to the clinical research unit at 7.30 AM. An indwelling cannula was inserted to allow blood sampling during the test. Two consecutive identical meals were served as breakfast at 09.00 AM and as lunch at 1.00 PM, each containing 905 kcal (50 g fat, 75 g carbohydrates, 35 g protein). Samples for determination of glucose, insulin, C-peptide, glucagon, glucagon-like peptide (GLP)-1 and glucose-dependent insulintropic polypeptide (GIP) were obtained at times 0, 5, 10, 20, 30, 60, 90, 120, 150, 180, 210 and 240 min following each meal, with the meal beginning immediately after the time 0 sample and being consumed within 15 min (Supplementary Figure 1C).

Heart Rate Variability

Prior to consumption of the first meal, continuous finger arterial blood pressure and heart rate were recorded (Portapres, FMS, Amsterdam, The Netherlands) in the supine position for 30 min on a beat-to-beat basis as described previously [21]. From the arterial pressure signal, interbeat
interval (IBI) was derived (Beatscope software1.1, FMS, Amsterdam, The Netherlands). Power spectral analysis was assessed by discrete Fourier transform as described previously [22]. The low-frequency band (LF; 0.04-0.15 Hz) and the high frequency band (HF; 0.15-0.4 Hz) were selected. LF and HF bands were expressed in normalized units (LF norm and HF norm, respectively), where HF norm represents parasympathetic activity. In addition, the LF/HF ratio was computed as a measure of sympathovagal balance [22]. Heart rate variability (HRV) derived from a finger plethysmograph (IBI) and ECG recordings (R-R interval) were shown to be highly comparable [23].

Study Medication
PRED tablets were purchased from Pfizer AB (Sollentuna, Sweden) and PLB tablets were obtained from Xendo Drug Development (Groningen, The Netherlands). Tablets were capsulated in order to allow the treatment to be blinded [8]. Study medication was taken at 08.00 AM during the two-week treatment except for day 13 and day 14, when it was ingested at 06.00 AM. Patients kept a diary in which the exact time of medication intake during the study was registered.

Analytical Determinations
Blood glucose concentrations were measured using an YSI 2300 STAT Plus analyzer (YSI, Yellow Springs, OH). Insulin and C-peptide levels were determined using an immunometric assay (Advia, Centaur, Siemens Medical Solutions Diagnostics, USA). Glucagon concentrations were determined by radioimmuno assay (Linco Research, St. Louis, USA). Total GLP-1 levels were measured using a C-terminally directed radioimmunoassay (antiserum no. 89390) as described previously [24]. Total GIP was analyzed using a newly developed assay, employing a C-terminally directed antiserum (code no 80867) raised in rabbits immunized with a C-terminal fragment of GIP [GIP (28-42)] conjugated to keyhole limpet haemocyanin via its N-terminus. This assay has broadly the same specificity and characteristics as the previously published assay using antiserum R65 [25], recognizing equally both intact GIP (1-42) and the primary metabolite, GIP (3-42).

Data Analyses
From the hyperglycemic clamp the beta-cell function parameters first-phase (min 0-10) and second-phase (min 10-80) incremental area under the C-peptide curve (iAUC CP), as well as arginine-stimulated C-peptide secretion (ASI- iAUC CP) (min 80-110) were calculated using the trapezoid method (nmol L⁻¹ min⁻¹). From the hyperinsulinemic-euglycemic clamp whole-body insulin sensitivity was quantified by the M-value (mg kg⁻¹ min⁻¹), calculated between min 90-120 during steady-state insulin concentrations as described previously [20]. During the double meal challenge absolute area under the curves (AUCs) for glucose, insulin, C-peptide, glucagon, GLP-1 and GIP were calculated using the trapezoid method.
Beta-cell Function during the Meal Challenge Test

Beta-cell function during the standardized double meal challenge test was assessed by mathematical modeling which was described in detail previously [26]. The model describes the relationship between insulin secretion and glucose concentration as the sum of two components. The first component represents the dependence of insulin secretion on absolute glucose concentrations at any time point and is characterized by a dose-response function relating the two variables. The characteristic parameter of the dose response is its mean slope, denoted here as glucose sensitivity. The dose response is modulated by both glucose-mediated and non-glucose-mediated factors (i.e. non-glucose substrates, gastrointestinal hormones, and neurotransmitters), which are collectively modeled as a potentiation factor. The excursion of the potentiation factor was quantified using a ratio between mean values at times 160-180 min and 0–20 min, and is called the potentiation factor ratio (PFR). In addition, the fasting secretory tone is calculated from the dose-response curve as insulin secretion at the glucose concentration of 4.5 mmol/L. The second component of the model describes the insulin response to the rate of change of glucose concentration. This component is termed rate sensitivity, which is related to early insulin release [26].

Statistical Analyses

Data are presented as mean values ± standard deviation (SD), or as median (interquartile range) in case of skewed distribution. Non-parametric analysis was chosen due to uneven sample size over the groups, the relatively small N, and unequal variances that were observed for some parameters. Between-group comparisons of baseline values were performed using Kruskal-Wallis test. For treatment-induced effects, absolute changes from baseline were calculated (on-treatment value minus pre-treatment value) and were compared by Kruskal-Wallis test with trend analysis (the Jonckheere-Terpstra test). Only in case of a significant finding, PRED7.5 and PRED30 were compared against placebo by posthoc testing, using the Mann-Whitney U test. To correct for multiple testing, Bonferroni correction was applied. Correlations between the various parameters were assessed with Pearson’s correlations.

The study was powered on the high-dose PRED group. We included 32 subjects (8 in the placebo group and 12 in each PRED arm), which provided us with approximately 85% power to detect changes of 30% with a SD of 25% (with alpha set at 0.05) of the different clamp-measured beta-cell parameters, including first- and second-phase C-peptide secretion adjusted for insulin sensitivity, and arginine-stimulated C-peptide secretion. No power calculation was made for the lower PRED dosage.

All statistical analyses were run on SPSS version 15 for Windows (Chicago, IL, USA). A P < 0.05 was considered statistically significant.
RESULTS

Anthropometric characteristics

No significant differences in subject characteristics were observed among the groups at baseline (Table 1). Body weight was not altered by PRED-treatment irrespective of the dose (Table 2). Systolic blood pressure (6±1.2 mmHg increase; \( P=0.006 \)), but not diastolic blood pressure was raised by PRED30. PRED7.5 did not affect blood pressure (Table 2).

Table 1. Subject characteristics at inclusion

<table>
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<tr>
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<th>Placebo</th>
<th>Prednisolone 7.5 mg</th>
<th>Prednisolone 30 mg</th>
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<td>8</td>
<td>12</td>
<td>12</td>
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<td>21±2</td>
<td>21±2</td>
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<td>184±6</td>
<td>185±5</td>
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<td>Systolic blood pressure (mmHg)</td>
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<td>79±6</td>
<td>78±10</td>
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<td>2-hr glucose OGTT (mmol/l)</td>
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<td>3.6±0.8</td>
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</table>

Data are mean±SD. No statistically significant differences were observed between the groups at baseline as tested by Kruskal-Wallis test. 2-hr glucose OGTT denotes plasma glucose concentrations 2 h after ingestion of 75 g glucose during an oral glucose tolerance test.

Fasting Glucose and Hormone Levels

A dose-dependent rise in fasting glucose levels was observed by PRED treatment relative to placebo \( (P=0.04) \) (Table 2). Fasting insulin levels were significantly increased \( (P=0.008) \) in the PRED30 arm. PRED30-induced increment in fasting insulin levels was due to increased basal secretion, not altered clearance, since fasting C-peptide levels were similarly enhanced \( (P=0.001) \) (Table 2).

Euglycemic Clamp

PRED treatment dose-dependently decreased insulin sensitivity (M-value) as compared to placebo: mean differences: \(-2.1±0.8 \text{ mg kg}^{-1} \text{ min}^{-1}\) for PRED7.5 and \(-4.5±0.7 \text{ mg kg}^{-1} \text{ min}^{-1}\) for PRED30 (Table 2). Insulin levels reached steady state during min 90-120 of the euglycemic clamp at 490±77 pmol/L prior to treatment, and were not altered during the on-treatment clamps (Table 2). Adjustment of the M-value by insulin levels during the steady-state part of the clamp \( (M/I) \) did not affect the results (data not shown).
Table 2. Metabolic and anthropometric parameters before and at day 14 of treatment

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<th>On-treatment</th>
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<th>P2</th>
<th>P3</th>
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<td>75±6</td>
<td>75±6</td>
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<td>30</td>
<td>73±10</td>
<td>74±10</td>
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<td>BMI (kg/m²)</td>
<td>PLB</td>
<td>21.6±1.5</td>
<td>21.7±1.3</td>
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<td>Systolic Blood Pressure (mmHg)</td>
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<td>7.5</td>
<td>124±9</td>
<td>124±11</td>
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<td>Diastolic Blood Pressure (mmHg)</td>
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<td>76±7</td>
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<td>4.5±0.3</td>
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<td>7.5</td>
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<tr>
<td>Fasting plasma insulin (pmol/l)</td>
<td>PLB</td>
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<td>33 (28-39)</td>
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<td></td>
<td>7.5</td>
<td>36 (29-47)</td>
<td>36 (27-65)</td>
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<tr>
<td></td>
<td>30</td>
<td>32 (26-44)</td>
<td>56 (41-72)</td>
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<tr>
<td>Fasting C-peptide (nmol/l)</td>
<td>PLB</td>
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<td>0.4±0.1</td>
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<td>7.5</td>
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<td>0.918</td>
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<td>0.4±0.1</td>
<td>0.6±0.2</td>
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<td>Fasting glucagon (pmol/l)</td>
<td>PLB</td>
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<td>10±3</td>
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<tr>
<td></td>
<td>7.5</td>
<td>12±3</td>
<td>13±3</td>
<td>0.002</td>
<td>0.152</td>
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<td></td>
<td>30</td>
<td>12±3</td>
<td>15±3</td>
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<tr>
<td>M-value (mg/kg.min)</td>
<td>PLB</td>
<td>7.0 (6.9-11.1)</td>
<td>9.0 (6.5-12.8)</td>
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<td></td>
<td>7.5</td>
<td>9.1 (7.4-10.6)</td>
<td>9.2 (4.6-11.1)</td>
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<td></td>
<td>30</td>
<td>7.9 (5.8-9.7)</td>
<td>4.4 (2.6-6.4)</td>
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<td>Clamp insulin levels (pmol/l)</td>
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<td></td>
<td>30</td>
<td>504±78</td>
<td>460±81</td>
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Data are mean ± SD or median (interquartile range). Between-group changes from baseline were tested by Kruskal-Wallis with trend analysis (indicated by P1). In case of a significant finding, posthoc testing by Mann-Whitney U with Bonferroni correction was done (P2 and P3, indicating PRED7.5 vs. placebo and PRED30 vs. placebo, respectively). Treatment groups: PLB=placebo; 7.5=prednisolone 7.5 mg daily; 30=prednisolone 30 mg daily.
Standardized Double Meal Challenge Test

PRED treatment dose-dependently increased AUC for glucose (AUC\textsubscript{G}) by 11±5% (\(P<0.008\)) and 27±9% (\(P<0.001\)), for PRED7.5 and PRED30 respectively (Figure 1A), despite dose-dependent increases in postprandial insulin and C-peptide levels (Figure 1B-C). Plasma levels of total GLP-1 and GIP were not changed by either of the PRED dosages (Figure 1D-E).

Figure 1. Postprandial responses at baseline and at 14 day of intervention. Panel A: Glucose concentrations. Panel B: Insulin concentrations. Panel C: C-peptide concentrations. Panel D: Glucagon-like peptide (GLP)-1 concentrations. Panel E: Glucose-dependent insulinoic polypeptide (GIP) concentrations. Panel F: Glucagon concentrations. Straight line with black squares denotes pre-treatment. Dotted line with open circles represents on-treatment. Box-and-Whisker plots with absolute change in area under the curve (AUC) from baseline are shown. Between-group changes from baseline were tested by Kruskal-Wallis test with trend analysis (indicated by top line). Posthoc tests were done by Mann-Whitney U with Bonferroni correction for multiple testing (indicated by line with brackets). ***\(P<0.001\); **\(P<0.01\).
Hyperglycemic Clamp
PRED treatment tended to increase first- and second-phase glucose-stimulated C-peptide secretion (Figure 2A, B). In multivariate analysis, however, this trend was no longer observed when the M-value was added in the model (1st-phase iAUC: \( \beta = 0.126; P = 0.638; R^2 = 0.119 \) and 2nd-phase iAUC: \( \beta = 0.051; P = 0.847; R^2 = 0.152 \)). C-peptide secretion following arginine stimulation on top of hyperglycemia (ASI-iAUC\(_{CP} \)) was significantly and dose-dependently reduced by PRED treatment: -2.7 (-5.2; -0.3) nmol L\(^{-1}\)min\(^{-1}\) for PRED\(_{7.5}\) and -3.0 (-7.6; -0.2) nmol L\(^{-1}\)min\(^{-1}\) for PRED\(_{30}\) (\( P = 0.035 \)) (Figure 2C). In multivariate analysis, this relation was independent of changes in insulin sensitivity. The results from the hyperglycemic clamp were similar when insulin iAUCs were calculated (data not shown).
Model-derived Parameters of Beta-cell Function
Neither PRED7.5 nor PRED30 affected beta-cell glucose sensitivity and rate sensitivity (Figure 2D-E). PFR was significantly decreased by PRED30, but not by PRED7.5 (Figure 2F). Basal insulin secretion at a fixed glucose level of 4.5 mmol/L tended to decrease following PRED treatment ($P=0.052$) (Figure 2G).

Glucagon Levels
Fasting glucagon levels were increased by PRED30 treatment, but not by PRED7.5 (Table 2). Similarly, only PRED30 ($P=0.004$) increased postprandial glucagon levels (Figure 1F).

Heart Rate Variability
Before treatment, LF/HF ratio in the entire study population was 1.42 (0.86-1.85) arbitrary units (AU), LF$_{norm}$ 42 (27-53) AU, and HF$_{norm}$ 33 (22-41) AU. PRED treatment did not affect LF/HF ratio, but PRED30 treatment showed a trend towards reduced HF$_{norm}$ by 14±5 AU as compared to PLB ($P=0.06$).

Associations between Heart Rate Variability and Islet-Cell Function
The change in HF$_{norm}$ was inversely associated with changes in FPG (Figure 3A) and tended to negatively associate with fasting glucagon levels ($\beta$=-0.337; $P=0.07$). The change in LH/FH ratio was inversely related with changes in ASI-iAUC$_{CP}$ ($\beta$=-365; $P=0.05$) (Figure 3B). There were no significant associations between measures of HRV and postprandial glucagon levels or measures of beta-cell function obtained during the meal test (data not shown).

Figure 3. Associations between heart rate variability and islet-cell function. Pearson correlations between changes from baseline in HF$_{norm}$ and fasting plasma glucose (A), and between changes from baseline in LF/HF ratio and arginine-stimulated C-peptide secretion (B).

Safety and Tolerability
One subject in the PRED30 group complained of sleeplessness, which was mild and transient of nature. Otherwise, no side effects were reported in all treatment arms.
DISCUSSION

The principal findings of the present study are that a two-week treatment with high-dose PRED impaired various parameters of pancreatic islet-cell function, and that altered ANS balance could be involved in these changes, although ANS balance itself was not significantly altered. Furthermore, in this study, we observed a clear dose-dependency of PRED-induced effects. However, probably due to lack of power, the effects of low-dose PRED treatment did not reach statistical significance. At increased levels, GCs induce glucose intolerance and diabetes in susceptible individuals, which has classically been attributed to GC-induced insulin resistance (4). Indeed, our study confirmed the presence of GC-induced insulin resistance: PRED treatment dose-dependently reduced clamp-measured M-value.

The effects of short-term GC treatment on islet-cell function, and particularly beta-cell function, have been under debate. The observation that 2 days GC exposure induced fasting hyperinsulinemia and increased insulin secretion in response to oral and intravenous glucose loads, may suggest a lowering of insulin sensitivity by GCs (9-13). In the present study, we similarly observed elevated fasting and postprandial insulin levels and a tendency towards increased first- and second-phase glucose-stimulated C-peptide secretion. Using multivariate analysis, we demonstrated in this study that increased 1st and 2nd phase C-peptide secretion was driven by treatment-related reduction in insulin sensitivity, showing adequate compensation for PRED-induced insulin resistance (Supplemental Figure 3A), and did not indicate improved beta-cell function per se. Interestingly, PRED treatment reduced arginine-stimulated C-peptide secretion, a measure of insulin secretory capacity, in a dose-dependent matter.

A similar pattern was observed during the meal challenge test. Modeling analysis of glucose and C-peptide concentrations, revealed decreased glucose-adjusted insulin secretion rates following PRED treatment, also at fasting glucose levels (Supplementary Figure 3B; Figure 4D for insulin secretion at 4.5 mmol/L glucose). While PRED treatment did not affect glucose sensitivity of the beta cell or rate sensitivity, PRED30 markedly decreased the potentiation factor ratio, a finding that confirms data from a previous study published by our group (8). This represents insulin secretion that is not primarily related to plasma glucose levels and may include secretion induced by non-glucose secretagogues, incretin hormones, and neuronal factors (27, 28).

From both the hyperglycemic clamp test and the meal test we conclude that PRED induces beta-cell dysfunction, and is detectable on specific beta-cell function parameters that involve potentiation phenomena (26, 28, 29). In addition to impairments in beta-cell function, we observed GC-induced increased fasting and postprandial glucagon levels during high-dose PRED treatment. Thus, PRED treatment altered islet-cell functional balance.
Additionally, we evaluated mechanisms possibly underlying the effects of GCs on islet-cell function. First, we evaluated the role of the ANS system. Whereas catecholamines released by the sympathetic nervous system inhibit insulin secretion via \( \alpha_2 \)-adrenergic receptor (AR) signaling and stimulate glucagon release, acetylcholine released by parasympathetic nerves stimulates insulin release via protein kinase C-related pathways [2]. In addition, parasympathetic nerve fibers increase islet blood flow, thus facilitating increased insulin secretion [30]. In beta-cell lines, GCs decreased the efficacy of acetylcholine to release insulin [5], and also upregulated expression and signaling of \( \alpha_2 \)-ARs [31]. Transgenic mice with beta-cell specific overexpression of the GR were shown to develop diabetes through beta-cell failure consequential to increased \( \alpha_2 \)-AR expression.

We observed a tendency towards withdrawal of vagal activity following high-dose PRED treatment, which was negatively associated with fasting glucose and glucagon levels. In addition, alterations in sympathovagal balance, expressed as LF/HF ratio, were negatively related to changes in C-peptide secretion in response to arginine. The latter finding is partly in line with a previous study, in which interruption of cholinergic transmission by trimethaphan impaired arginine-stimulated insulin secretion during treatment with dexamethasone [32]. However, in that acute study, a 2-day treatment with high-dose dexamethasone, increased arginine-stimulated secretion, whereas we observed a decline in this beta-cell function parameter. Differences in treatment duration and study population (in that study, highly insulin sensitivity participants, as determined by hyperinsulinemic-euglycemic clamp prior to inclusion, were studied) may have contributed to these seemingly opposing results. In addition, dexamethasone is a pure GR agonist, whereas PRED also activates the mineralocorticoid receptor. Various GC compounds, depending on their receptor specificity, may have a different effect on measures of cardiovascular autonomic function and variables such as blood pressure and heart rate, making the compounds difficult to compare [33, 34]. It should be stressed that in the present experimental design, we were unable to measure the direct effects of changes in ANS balance on islet-cell function, and, based on observed correlations, present a hypothesis. This hypothesis needs to be explored in future experiments, leaving the role of autonomic dysfunction in GC-induced islet-cell dysfunction presently unanswered.

Second, we measured incretin responses following PRED treatment. The incretin hormones GLP-1 and GIP substantially contribute to glucose tolerance and we hypothesized that PRED treatment would reduce incretin levels [35]. However, we did not observe declined plasma levels of GLP-1 and GIP following PRED treatment, confirming a recently published study, in which high-dose PRED treatment combined with a hypercaloric diet and physical inactivity did not decrease postprandial levels of GLP-1 and GIP [15]. However, the same authors demonstrated in a subsequent study that the same intervention reduced the insulinotropic effects of GLP-1 and GIP when infused at
Thus, an impaired incretin effect at the level of the beta cell may in fact contribute to GC-induced hyperglycemia. Future in vitro studies will need to address this hypothesis.

Recently, we investigated the role of endoplasmic reticulum (ER) stress in GC-induced beta-cell dysfunction. We observed in INS-1E cells that PRED treatment impaired the function of the ER contributing to impaired insulin secretion, reduced insulin biosynthesis and increased expression of apoptotic markers. It remains to be established which pathways within the beta cell are most importantly affected by GC treatment.

Our study is strengthened by the design of a randomized controlled clinical trial, and treatment duration of two weeks, allowing to investigate past the (sub)acute effects of GC treatment on islet-cell function. In addition, we included a clinically relevant low- and high-dosage to study dose-dependency. However, our study has a number of limitations. First, our study is of relatively short duration when compared to GC treatment duration in clinical practice, especially for the low dosage which is sometimes continued for years. In addition, patients in the clinic that are treated with GC are usually characterized by systemic inflammation, reduced insulin sensitivity and impaired pancreatic islet-cell function. In addition, our study has a number of limitations. Finally, the possible role of altered ANS balance in GC-induced islet-cell function remains presently unanswered since our observations are based on correlations.

Nevertheless, the results obtained in the present study may have important implications for the treatment of GC-induced glucose intolerance and diabetes. Our data indicate that pharmacological measures that aim to improve pancreatic islet-cell function may particularly be effective in restoring glucose tolerance during PRED treatment. In line with this hypothesis, we have recently shown that GC-induced glucose intolerance could be prevented by concurrent treatment with the GLP-1 receptor agonist exenatide when infused at pharmacological levels [7]. Further studies in relevant populations should explore the full potential of incretin-based therapies to prevent GC-induced glucose intolerance.

We conclude that, in addition to reducing insulin sensitivity, prolonged PRED treatment dose-dependently impairs islet-cell function in healthy males. We furthermore suggest that changes in ANS balance could contribute to these GC-related changes.
CHAPTER 2

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REFERENCES


