Chapter 4

Acute and 2-Week Exposure to Prednisolone Impair Different Aspects of Beta-Cell Function in Healthy Men

ABSTRACT

Objective. Glucocorticoids (GCs), such as prednisolone, are associated with adverse metabolic effects, including glucose intolerance and diabetes. In contrast to the well-known GC-induced insulin resistance, effects of GCs on beta-cell function are less well established. We assessed the acute and short-term effects of prednisolone treatment on beta-cell function in healthy men.

Research Design and Methods. A randomized, double blind, placebo-controlled trial, consisting of two protocols was conducted. In protocol 1 (n=6), placebo and a single dose of 75 mg prednisolone were administered. In protocol 2 (n=23), participants received 30 mg prednisolone daily or placebo for 15 days. Both empirical and model-based parameters of beta-cell function were calculated from glucose, insulin and C-peptide concentrations obtained during standardized meal tests before and during prednisolone treatment (protocols 1 and 2), and one day after cessation of treatment (protocol 2).

Results: 75 mg prednisolone acutely increased the area under the postprandial glucose curve (AUCg) (P=0.005) and inhibited several parameters of beta-cell function, including AUCcpep/AUCg ratio (P=0.004), insulinogenic index (P=0.007), glucose sensitivity (P=0.02), and potentiation factor ratio (PFR) (P=0.04). A 15-day treatment with prednisolone increased AUCg (P=0.001), despite augmented C-peptide secretion (P=0.05). Beta-cell function parameters were impaired, including the fasting insulin secretory tone (P=0.02) and PFR (P=0.007).

Conclusions: Acute and short-term exposure to prednisolone impair different aspects of beta-cell function, which contribute to its diabetogenic effects.

Clinical Trial Registration Number: NCT00971724
Glucocorticoids (GCs), such as prednisolone, are very efficacious and frequently prescribed anti-inflammatory drugs. Unfortunately, supraphysiological levels of GCs induce adverse metabolic effects, including glucose intolerance and diabetes [1]. Steroid diabetes may develop in up to 20-50% of patients with excessive plasma GC levels [2]. GCs are well known to reduce insulin sensitivity, resulting in increased hepatic glucose production and decreased peripheral glucose disposal [3]. The role of beta-cell dysfunction in GC-related diabetogenic effects is less clear. GCs impaired insulin secretion in rodent-derived islets in vitro [4]. In vivo in both rodents [5] and humans [6], a single day of GC administration impaired insulin secretion, resulting in hyperglycemia. More prolonged exposure to GCs, on the other hand, induced fasting hyperinsulinemia and increased insulin secretion in both wild type rodents [7] and healthy humans [8-12], most likely to compensate for impaired insulin sensitivity. In rodent models of obesity [13, 14] and in susceptible humans, however, this compensation failed. These ‘at risk’ populations included normoglycemic individuals with reduced insulin sensitivity or low glucose-stimulated insulin secretion (GSIS) before GC treatment [9, 11, 12] and normoglycemic, first-degree relatives of patients with type 2 diabetes mellitus [10]. It was concluded that GCs may only induce beta-cell dysfunction in vulnerable populations.

The above-mentioned studies, however, have a limitation. Beta-cell function was assessed by tests using intravenous glucose loads, such as the intravenous glucose tolerance test (IVGTT) or the hyperglycemic clamp. As the magnitude of the insulin response under normal conditions also depends on other factors, such as non-glucose substrates [15, 16], incretins [17] and neurotransmitters [18], the hyperglycemic clamp may represent a less physiological condition relative to tests using orally administered insulin secretagogues.

More recently, various parameters of beta-cell function have been calculated by modeling glucose and C-peptide plasma concentrations during standardized meal tests [19]. This approach enables the assessment of various aspects of beta-cell function under daily life conditions and also allows to evaluate the separate roles of insulin secretion and insulin sensitivity on glucose control in a single test [19].

The aim of the present study was to assess the effects of both acute and short-term exposure to a widely used GC, i.e. prednisolone, on various aspects of beta-cell function in healthy men.
RESEARCH DESIGN AND METHODS

Study design: The study was a single-center, double blind, randomized, placebo-controlled study, consisting of two distinct parts.

Protocol 1: Acute Study: In order to assess the acute effects of prednisolone treatment, eligible participants (n=6) ingested a placebo tablet on day 0 at 08:00 AM and a 75 mg prednisolone capsule at 08:00 AM on day 1. No study medication was given on day 2. Standardized meal tests were performed on days 0, 1 and 2 at 10:00 AM.

Protocol 2: Two-week study: The effects of short-term treatment with prednisolone on beta-cell function were assessed in different subjects. Participants (n=23) were randomly assigned to a treatment with either 30 mg prednisolone once daily (n=12) or placebo (n=11) for a period of 15 days (medication was taken in the morning). Standardized meal tests were performed at day 0 and at day 15 at 10:00 AM. Placebo was administered as a subject-blinded treatment on day 0 at 08:00 AM (baseline). On day 15, study medication was also administered at 08:00 AM.

Study medication: Prednisolone tablets were obtained from Pfizer AB (Sollentuna, Sweden), placebo tablets were provided by Schering-Plough (Oss, The Netherlands). The tablets were encapsulated in order to allow the treatment to be blinded.

Study population: Both protocols enrolled healthy male volunteers (age range 20 and 45 years; body mass index (BMI) 22-30 kg/m²). Health status was confirmed by medical history taking, physical and laboratory examinations, and ECG and vital signs recordings. Furthermore, normal glucose metabolism was verified by a 75 g 2-hour oral glucose tolerance test (OGTT). Participants were excluded if they had a clinically relevant history or presence of a medical disorder known to affect the investigational parameters, if they were taking medication, except for incidental analgesics, or if they had a first-degree relative with type 2 diabetes mellitus.

Study assessments: Screening assessments were performed within a three-week period prior to inclusion. Subjects were admitted to the clinical research unit at Xendo Drug Development (Groningen, The Netherlands) at 18:00 PM. The following day at 10:00 AM, subjects consumed a standardized 4-hour meal test (35 g proteins, 39 g fat and 75 g carbohydrates) after an overnight fast of minimal 12 hours. Samples for determination of glucose, insulin and C-peptide were obtained at times 0, 5, 10, 20, 30, 60, 90, 120, 180, and 240 min, with the
meal beginning immediately after the time 0 sample and consumed within 15 min. Insulin and C-peptide were measured by Xendo Drug Development using immuno assays (Mercodia, Uppsala, Sweden).

**Data analysis:** Area under the 4-hr postprandial glucose (AUC_{gluc}) and C-peptide (AUC_{c-pep}) curves were determined by using the trapezoidal rule. Measures of insulin sensitivity, including homeostatic model assessment (HOMA-IR) [20] and oral glucose insulin sensitivity (OGIS) [21] were calculated. Empirical measures of beta-cell function, including AUC_{c-pep}/AUC_{gluc} ratio and the insulinogenic index (IGI) (insulin_{t=30}-insulin_{t=0})/(gluc_{t=30}-gluc_{t=0}) [22] were calculated.

**Modeling analysis of beta-cell function:** Pancreatic beta-cell function was assessed with a model that describes the relationship between insulin secretion and glucose concentration, which has been described in detail previously [19]. The model expresses insulin secretion (in pmol/min per m² of body surface area) as the sum of two components. The first component describes the dose-response relation between insulin secretion and glucose concentrations during the meal test. Three parameters are obtained from this dose-response relation. First, the sensitivity of the beta cell to changes in plasma glucose levels, called glucose sensitivity. It is derived from the mean slope of the dose-response curve. Second, the fasting secretory tone is calculated from the dose-response curve. This represents fasting insulin secretion rates at a fixed glucose concentration of 4.5 mM (approximately the mean fasting glucose concentration in the groups). The third parameter is a potentiation factor, which may account for several potentiating signals to the beta cell (e.g. non-glucose metabolites, incretin hormones and neural factors) or amplifying pathways within the beta cell [23], although its exact physiological basis warrants further investigation. The excursion of the potentiation factor was quantified using a ratio between mean values at times 160-180 and 0–20 min, and is called the potentiation factor ratio (PFR). 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 [19].

The model parameters were estimated from glucose and C-peptide concentration by regularized least squares, as described previously. Regularization involves the choice of smoothing factors that were selected to obtain glucose and C-peptide model residuals with standard deviations close to the expected measurement error (1% for glucose and 5% for C-peptide). Insulin secretion rates (ISR) were calculated from the model every 5 min [24]. Estimation of the individual model parameters was performed blinded to the randomization of patients to treatment.
Statistical analysis: Data are presented as mean values ± standard error of the mean (SEM) or, in case of skewed distribution, as median (interquartile range). In the acute study, outcome variables were log-transformed and differences were tested by analysis of variance (ANOVA) with Bonferroni post-hoc test. For the two-week study, between-group treatment effects were tested with Mann-Whitney U. All statistical analyses were run on SPSS for Windows version 15.0 (SPSS, Chicago, IL, USA). A \( P < 0.05 \) was considered statistically significant.

Ethics and good clinical practice: All participants provided written informed consent. The study was approved by an independent ethics committee and the study was conducted in accordance with the Declaration of Helsinki, using good clinical practice.

RESULTS

Acute study

Fasting metabolic parameters and glucose- and C-peptide profiles during meal tests:

Six healthy participants were included in this protocol (age: 24.3 ±1.5 years, BMI: 24.2 ± 0.9 kg/m\(^2\)). High single-dose prednisolone treatment did not change fasting plasma glucose (FPG) or insulin (FPI), but decreased OGIS (\( P=0.009 \)) (Table 1). During the meal test, prednisolone augmented AUC\(_{\text{gluc}}\) (\( P=0.005 \)), while C-peptide secretion remained unchanged (Figure 1, panels A and B; Table 1). One day following discontinuation of prednisolone treatment, glucose levels during the meal test normalized, but AUC\(_{\text{c-pep}}\) was increased (\( P=0.002 \)).

Figure 1. 75 mg Prednisolone acutely increased postprandial glucose concentrations (panel A), which was not accompanied by increased AUC\(_{\text{c-pep}}\) (panel B). After discontinuation of prednisolone treatment, glucose concentrations normalized during the meal test, but C-peptide levels were increased. Solid line with black squares represents day 0 (placebo), dotted line with black circles denotes day 1 (prednisolone 75 mg), and intersected line with white circles is day 2 (no treatment). Data are mean ± SEM.
Table 1 (Acute study). Subject characteristics following administration of placebo (day 0) and 75 mg prednisolone (day 1), and after cessation of treatment (day 2). Values are median (interquartile range). P1 indicates day 0 versus day 1; P2 denotes day 1 versus day 2.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 2</th>
<th>P1</th>
<th>P2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>4.6 (4.1-4.8)</td>
<td>4.8 (4.3-5.2)</td>
<td>4.2 (3.8-4.3)</td>
<td>NS</td>
<td>0.037</td>
</tr>
<tr>
<td>Fasting plasma insulin (pmol/l)</td>
<td>22 (21-27)</td>
<td>26 (22-31)</td>
<td>29 (22-33)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>HOMA-IR (no dimension)</td>
<td>0.5 (0.5-0.6)</td>
<td>0.6 (0.5-0.7)</td>
<td>0.6 (0.5-0.7)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>OGIS (ml/min/m²)</td>
<td>459 (424-509)</td>
<td>387 (341-418)</td>
<td>490 (470-503)</td>
<td>0.009</td>
<td>0.003</td>
</tr>
<tr>
<td>AUC_{gluc} (mmol/L.240min)</td>
<td>1210 (966-1295)</td>
<td>1565 (1393-1776)</td>
<td>1264 (1033-1309)</td>
<td>0.005</td>
<td>0.017</td>
</tr>
<tr>
<td>AUC_{cpep} (nmol/L.240min)</td>
<td>37 (32-41)</td>
<td>34 (30-40)</td>
<td>50 (48-57)</td>
<td>NS</td>
<td>0.003</td>
</tr>
<tr>
<td>Insulinogenic Index (no dimension)</td>
<td>173 (131-303)</td>
<td>74 (55-94)</td>
<td>174 (96-217)</td>
<td>0.007</td>
<td>0.04</td>
</tr>
<tr>
<td>AUC_{cpep}/AUC_{gluc} x 1000 (no dimension)</td>
<td>30 (26-36)</td>
<td>21 (19-26)</td>
<td>43 (40-47)</td>
<td>0.004</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Abbreviations. AUC_Glc: area under postprandial glucose curve; AUC_c-pep: area under postprandial C-peptide curve; HOMA-IR: homeostatic model assessment insulin resistance; OGIS: oral glucose insulin sensitivity.

Parameters of beta-cell function: A single dose of 75 mg prednisolone reduced several beta-cell function parameters as compared to placebo. The AUC_{cpep}/AUC_{gluc} ratio and IGI decreased with 29 ± 9% (P=0.002) and 59 ± 15% (P=0.007), respectively (Table 1). The model-derived parameters glucose sensitivity (41 ± 21% reduction; P=0.02) and PFR (39 ± 26% reduction; P=0.04) were impaired, rate sensitivity and fasting secretory tone, however, were non-significantly reduced. All parameters of beta-cell function were recovered on day 2 (Figure 2, panels A-D).
Figure 2. 75 mg Prednisolone reduced all model-derived parameters of beta-cell function, of which glucose sensitivity and potentiation factor ratio reached statistical significance. Graphs are Box-and-Whisker plots with median and interquartile range. Panel A: glucose sensitivity of the beta cell; panel B: basal secretory tone; panel C: potentiation factor ratio; panel D: rate sensitivity. * P<0.05; ** P<0.01.

Two-week study

Fasting metabolic parameters and glucose- and C-peptide profiles during the meal tests: Twenty-three healthy males were included in this protocol (age: 26.3 ± 1.1 years, BMI: 24.1 ± 0.4 kg/m²). Body weight was not affected by prednisolone treatment. Prednisolone treatment increased FGP (P=0.023) and decreased HOMA-IR (P=0.06) and OGIS (P=0.031) (Table 2). During standardized meal tests, a 15-day treatment with prednisolone increased AUC\textsubscript{gluc} (P < 0.001), despite augmented C-peptide secretion (P=0.05) (Figure 3, panels A-D; Table 2).

Parameters of beta-cell function: Prednisolone treatment impaired the model-based parameters fasting insulin secretory tone (P=0.02) and PFR (P=0.007), but did not affect IGI, AUC\textsubscript{ISR}/AUC\textsubscript{gluc} ratio, glucose sensitivity and rate sensitivity (Figure 4, panels A-D; Table 2). In multiple regression analysis, the PFR and OGIS similarly affected the change in AUC\textsubscript{gluc} during the meal tests (standardized correlation coefficients -0.482 and -0.500 for PFR and OGIS respectively).
Figure 3. A two-week treatment with prednisolone increased area under the postprandial glucose curve (panel A: placebo; panel B: prednisolone), despite increased C-peptide levels (panel C: placebo; panel D: prednisolone). Solid line with black squares represents day 0, dotted line with white squares represents day 15. Data are mean ± SEM.

Figure 4. A 15-day treatment with prednisolone decreased the basal secretory tone and potentiation factor ratio (panel B and C). Glucose sensitivity and rate sensitivity were not affected (panel A and D). Graphs are Box-and-Whisker plots with median and interquartile range. * P<0.05; ** P<0.01.
**DISCUSSION**

GCs are well known to perturb glucose metabolism in humans, which most often is attributed to reduction of insulin sensitivity. It is far less clear to what extent beta-cell dysfunction contributes to the diabetogenic effects of GC therapy. In this study, we demonstrate that...
prednisolone, as the most widely prescribed oral GC worldwide, in addition to reducing insulin sensitivity, impairs beta-cell function in healthy men, both following acute and two-week exposure. In contrast to previous studies, we assessed the effects of GCs on beta-cell function under daily life conditions, i.e. during standardized meal tests [19].

A single dose of 75 mg prednisolone acutely increased AUC_{gluc}, while C-peptide secretion failed to respond (Figure 1, panels A and B). These data confirm and expand previous studies assessing the acute effects of GCs in both rodents [5] and humans [6], in which a single-day treatment with hydrocortisone [5] or prednisolone [6] prevented adequate response of beta cells to hyperglycemia. In line with these observations, Plat and colleagues demonstrated that elevation of morning cortisol levels acutely impairs insulin secretion [25]. In our study, all measured beta-cell function parameters declined by 25-50%, including measures for early- and late phase insulin secretion. In vitro studies in rodent islet cells have revealed several mechanisms by which GCs acutely interfere with insulin secretory pathways. First, GCs reduce the uptake and oxidation of several metabolites including glucose. Moreover, GCs augment outward potassium currents, which, by reducing cell membrane depolarization, limit calcium influx. In addition, GCs may reduce insulin secretion by decreasing the efficacy of calcium on the secretory process. Finally, GCs were shown to reduce insulin secretion induced by the parasympathetic nervous system [4, 26].

On day 2, beta-cell function appeared to have recovered from the acute effects of prednisolone, since fasting insulin secretion and insulin secretion during the standardized meal test were increased. This may indicate delayed compensation for the insulin resistance on day 1, but the increased insulin secretion could also serve to correct subtle disturbances in glucose homeostasis, although surrogate markers for insulin sensitivity were not decreased on day 2. In contrast to the acute study, subjects treated with prednisolone 30 mg daily for 15 days, increased C-peptide secretion during prednisolone treatment (Figure 3). Despite this enhanced secretion, however, fasting glucose and postprandial AUC_{gluc} exceeded baseline levels, indicating a relative hypoinsulinemia and accordingly a decline in beta-cell function. This relative hypoinsulinemia under fasting conditions becomes evident in our beta-cell model parameter ‘insulin secretory tone at a glucose level of 4.5 mM’, in which ISR are directly related to glucose plasma concentrations. This parameter was significantly reduced following 15-days of prednisolone exposure. In addition to insufficient basal secretory tone, PFR was also reduced by a two-week prednisolone treatment.

Previous studies in which subjects were exposed for multiple days to GCs, also reported increased insulin secretion, as assessed by hyperglycemic clamps [8, 9, 11, 12] and IVGTTs
However, the studies that accounted for GC-induced reductions in insulin sensitivity by calculating the disposition index [11] or by using minimal model analysis [10], reported adequate compensation of insulin resistance in healthy subjects through sufficiently augmented insulin secretion. Only subjects with (subtle) glucometabolic abnormalities prior to GC treatment were unable to fully compensate for GC-induced insulin resistance [9-12]. The most important difference between the above-mentioned studies and our study is that we used an oral stimulation test to assess various aspects of beta-cell function. Our experimental design may be more physiological compared to tests using intravenous glucose, since the former comprises the contribution of multiple factors, including incretins [17], non-glucose metabolic stimuli, such as non-esterified fatty acids (NEFA) [15] or amino acids [16], and the autonomic nervous system [18], all of which together account for a substantial proportion of the normal meal-related insulin response. These non-glucose insulin secretagogues are included as the ‘potentiation factor’ in our beta-cell model, which was significantly impaired by prednisolone, both following acute and short-term exposure. Our findings illustrate that the harmful effects of GCs on beta-cell function may only become fully apparent when using an oral stimulation test, which more comprehensively tests the role of the intestinal-islet axis on glucose homeostasis. To identify the specific non-glucose stimuli for insulin secretion that are impaired by GC treatment will require additional investigation.

It is important to note that the meal-induced insulin response during prednisolone treatment was markedly different in the acute protocol as compared to the two-week study. We propose that GCs induce an acute inhibitory effect on beta-cell function, as extensively demonstrated in both in vitro and in vivo experiments, but that beta-cell function partly recovers following more prolonged exposure. In the latter situation, GC-induced insulin resistance may oppose the direct effect of GCs on the beta cell by enhancing insulin secretion. It should however also be stressed that the dosages used in the two protocols were different, which could have contributed to the observed difference in insulin responses. It is well known that the effects of GCs on glucose metabolism are highly dependent on the administered dose [27].

We conclude that GCs impair several aspects of beta-cell function, both following acute and short-term treatment, in healthy normoglycemic men, when measured under physiological, daily life conditions. These data indicate that GC-induced beta-cell dysfunction, in addition to insulin resistance, contributes to the development of steroid diabetes.

**Funding:** This paper was written within the framework of project T1-106 of the Dutch Top Institute Pharma.
REFERENCES


