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# 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/m$^2$). 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).

*Abbreviations:* ANS, autonomic nervous system; ASI-iAUC, arginine-stimulated incremental area under the curve; AR, adrenergic receptor; AU, arbitrary units; BMI, body mass index; FPG, fasting plasma glucose; GC, Glucocorticoid; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; GSIS, glucose-stimulated insulin secretion; HF, high frequency; HRV, heart rate variability; GR, glucocorticoid receptor; iAUC, incremental area under the curve; IBI, interbeat interval; LF, low frequency; OGTT, oral glucose tolerance test; PFR, potentiation factor ratio; PLB, placebo; PRED, prednisolone.

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*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.



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 $\alpha$-2 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 (a 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 ratios was 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-dose PRED and high-dose PRED, a very commonly prescribed GC compound, were administered for a period of two weeks.

## 1. Research Design and Methods

### 1.1. *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/m$^2$, 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 75 g 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.

### 1.2. *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