

CLINICAL STUDY

Acute and 2-week exposure to prednisolone impair different aspects of β -cell function in healthy men

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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, the effects of GCs on β -cell function are less well established. We assessed the acute and short-term effects of prednisolone treatment on β -cell function in healthy men.

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

Results: Seventy-five milligrams of prednisolone acutely increased the area under the postprandial glucose curve (AUC_{gluc} ; $P=0.005$), and inhibited several parameters of β -cell function, including $AUC_{\text{c-pep}}/AUC_{\text{gluc}}$ 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 AUC_{gluc} ($P<0.001$), despite augmented C-peptide secretion ($P=0.05$). β -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 impairs different aspects of β -cell function, which contribute to its diabetogenic effects.

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Introduction

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 β -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 hyperglycaemia. More prolonged exposure to GCs, on the other hand, induced fasting hyperinsulinaemia 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 normoglycaemic individuals with reduced insulin sensitivity or low glucose-stimulated insulin secretion before GC treatment (9, 11, 12) and normoglycaemic, first-degree relatives of patients with type 2 diabetes mellitus (10). It was concluded that GCs may only induce β -cell dysfunction in vulnerable populations.

The above-mentioned studies, however, have a limitation. β -cell function was assessed by tests using i.v. glucose loads, such as the i.v. glucose tolerance test (IVGTT) or the hyperglycaemic 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 hyperglycaemic clamp may represent a less physiological condition relative to tests using orally administered insulin secretagogues.

More recently, various parameters of β -cell function have been calculated by modelling glucose and C-peptide plasma concentrations during standardised meal tests (19). This approach enables the assessment of various aspects of β -cell function under daily life conditions and also allows evaluation of 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 β -cell function in healthy men.

Research design and methods

Study design

The study was a single-centre, double-blind, randomised, 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 0800 h and a 75-mg prednisolone capsule on day 1 at 0800 h. No study medication was given on day 2. Standardised meal tests were performed on days 0, 1 and 2 at 1000 h.

Protocol 2: 2-week study The effects of short-term treatment with prednisolone on β -cell function were assessed in different subjects. Participants ($n=23$) were randomly assigned to a treatment with either 30 mg of prednisolone once daily ($n=12$) or placebo ($n=11$) for a period of 15 days (medication was taken in the morning). Standardised meal tests were performed at day 0 and at day 15 at 1000 h. Placebo was administered as a subject-blinded treatment on day 0 at 0800 h (baseline). On day 15, study medication was also administered at 0800 h.

Prednisolone tablets were obtained from Pfizer AB (Sollentuna, Sweden), and 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 electrocardiography (ECG) and vital signs recordings. Furthermore, normal glucose metabolism was verified by a 75-g 2-h oral glucose tolerance test.

Participants were excluded if they had a clinically relevant history or presence of a medical disorder known to affect the investigational parameters, were taking medication, except for incidental aspirin, or if they had a first-degree relative with type 2 diabetes mellitus.

Study assessments

Screening assessments were performed within a 3-week period prior to inclusion. Subjects were admitted to the clinical research unit at Xendo Drug Development (Groningen, The Netherlands) at 1000 h. The following day at 1000 h, subjects consumed a standardised 4-h meal (35 g proteins, 39 g fat and 75 g carbohydrates) after an overnight fast of a minimum of 12 h. 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 immunoassays (Mercodia, Uppsala, Sweden).

Data analysis

Area under the 4-h postprandial glucose (AUC_{gluc}) and C-peptide ($AUC_{\text{c-pep}}$) curves was determined by using the trapezoidal rule. Measures of insulin sensitivity including homeostatic model assessment of insulin resistance (HOMA-IR) (20) and oral glucose insulin sensitivity (OGIS) (21) were calculated. Empirical measures of β -cell function including $AUC_{\text{c-pep}}/AUC_{\text{gluc}}$ ratio and the insulinogenic index (IGI) ($\text{insulin}_{t=30} - \text{insulin}_{t=0} / (\text{gluc}_{t=30} - \text{gluc}_{t=0})$) (22) were calculated.

Table 1 Subject characteristics following administration of placebo (day 0) and 75 mg prednisolone (day 1), and after cessation of treatment (day 2).

	Pretreatment (day 0)	Prednisolone 75 mg (day 1)	Off-drug (day 2)	P^*	P^\dagger
FPG (mmol/l)	4.6 (4.1; 4.8)	4.8 (4.3; 5.2)	4.2 (3.8; 4.3)	NS	0.037
FPI (pmol/l)	22 (21; 27)	26 (22; 31)	29 (22; 33)	NS	NS
HOMA-IR	0.5 (0.5; 0.6)	0.6 (0.5; 0.7)	0.6 (0.5; 0.7)	NS	NS
OGIS (ml/min per m ²)	459 (424; 509)	387 (341; 418)	490 (470; 503)	0.009	0.003
AUC_{gluc} (mmol/l per min)	1210 (966; 1295)	1565 (1393; 1778)	1264 (1033; 1309)	0.005	0.017
$AUC_{\text{c-pep}}$ (nmol/l per min)	37 (32; 41)	34 (30; 40)	50 (48; 57)	NS	0.003
IGI	173 (131–303)	74 (55; 94)	174 (96; 217)	0.007	0.04
$AUC_{\text{ISR}}/AUC_{\text{gluc}} \times 1000$	30 (26; 36)	21 (19; 26)	43 (40; 47)	0.004	<0.0001

Values are median (interquartile range). Significance was tested with ANOVA with Bonferroni correction (after log-transformation of variables). P^* indicates day 0 versus day 1; P^\dagger indicates day 1 versus day 2.

Modelling analysis of β -cell function

Pancreatic β -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 m^2 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. The first parameter is the sensitivity of the β -cell to changes in plasma glucose levels called glucose sensitivity. It is derived from the mean slope of the dose–response curve. The second parameter is the fasting secretory tone, which is calculated from the dose–response curve. This represents fasting insulin secretion rates (ISRs) 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 β -cell (e.g. non-glucose metabolites, incretin hormones and neural factors) or amplifying pathways within the β -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 regularised least squares, as described previously. Regularization involves the choice of smoothing factors that were selected to obtain glucose and C-peptide model residuals with s.d.s close to the expected measurement error (1% for glucose and 5% for C-peptide). ISRs 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 \pm s.e.m. or, in case of skewed distribution, as median (interquartile range). In the acute study, outcome variables were log-transformed, and differences were tested by ANOVA with Bonferroni post-hoc test. For the 2-week study, between-group treatment effects were tested with Mann–Whitney *U* test. 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 or insulin, but decreased OGIS ($P = 0.009$; Table 1). During the meal test, prednisolone augmented AUC_{gluc} ($P = 0.005$), while C-peptide secretion remained

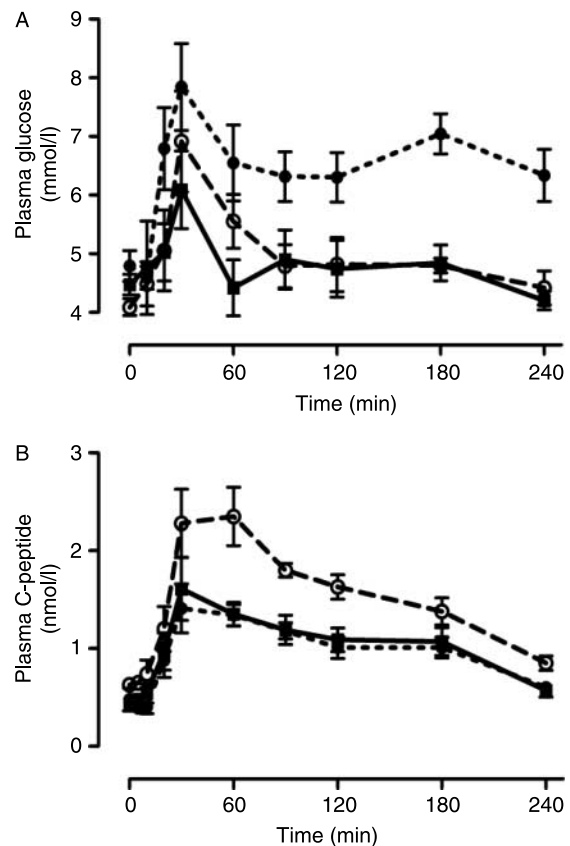


Figure 1 Seventy-five milligrams of prednisolone acutely increased postprandial glucose concentrations (panel A), which was not accompanied by increased AUC_{C-pep} (panel B). After discontinuation of prednisolone treatment, glucose concentrations normalised during the meal test, but C-peptide levels were increased. Solid line with black squares represents day 0 (placebo), dotted line with black circles denotes day 1 (75 mg of prednisolone) and intersected line with white circles represents day 2 (no treatment). Data are mean \pm s.e.m.

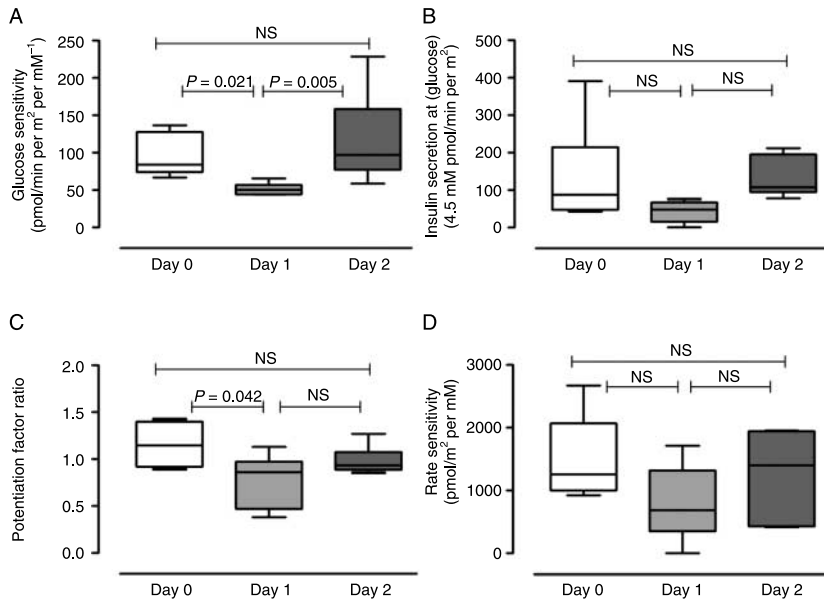


Figure 2 Seventy-five milligrams of prednisolone reduced all model-derived parameters of β -cell function, of which glucose sensitivity and potentiation factor ratio reached statistical significance. Graphs are box and whisker plots with median and interquartile range. Glucose sensitivity of the β -cell (panel A); basal secretory tone (panel B); potentiation factor ratio (panel C); rate sensitivity (panel D).

unchanged (Fig. 1, panels A and B; Table 1). One day after discontinuation of prednisolone treatment, glucose levels during the meal test normalised, but AUC_{c-pep} was increased ($P=0.002$).

Parameters of β -cell function A single dose of 75 mg of prednisolone reduced several β -cell function parameters as compared to placebo. The AUC_{c-pep}/AUC_{gluc}

ratio and IGI decreased with $29 \pm 9\%$ ($P=0.002$) and $59 \pm 15\%$ ($P=0.007$) respectively (Table 1). The model-derived parameters glucose sensitivity ($41 \pm 21\%$ reduction; $P=0.02$) and PFR ($39 \pm 26\%$ reduction; $P=0.04$) were impaired; however, rate sensitivity and fasting secretory tone were non-significantly reduced. All parameters of β -cell function were recovered on day 2 (Fig. 2, panels A–D).

Table 2 Subject characteristics before and during 15-day treatment with 30 mg prednisolone daily ($n=12$) or placebo ($n=11$).

	Pretreatment (day 1) Median (IQR)	On-drug (day 15) Median (IQR)	Change from pretreatment Median (IQR)	<i>P</i>
FPG (mmol/l)				
Placebo	4.6 (4.5; 4.9)	4.4 (3.9; 4.8)	-0.2 (-0.6; 0.3)	
Prednisolone	4.3 (3.9; 5.2)	5.0 (4.4; 5.1)	0.5 (0.0; 0.8)	0.023
FPI (pmol/l)				
Placebo	22 (21; 36)	27 (22; 37)	2.5 (-2.6; 5.5)	
Prednisolone	28 (22; 39)	57 (39; 68)	24.9 (-1.6; 37.8)	NS
HOMA-IR				
Placebo	0.5 (0.4; 0.8)	0.6 (0.5; 0.7)	0.0 (0.0; 0.1)	
Prednisolone	0.6 (0.5; 0.8)	1.2 (0.8; 1.5)	0.6 (0.0; 0.8)	0.06
OGIS (ml/min per m ²)				
Placebo	463 (441; 524)	496 (444; 517)	9.7 (-30; 59)	
Prednisolone	457 (427; 511)	404 (374; 415)	-50.2 (-104; -12)	0.031
AUC_{gluc} (mmol/l per min)				
Placebo	1173 (1038; 1241)	1147 (1097; 1224)	45 (-94; 112)	
Prednisolone	1224 (1070; 1297)	1466 (1389; 1532)	286 (156; 360)	0.001
AUC_{c-pep} (nmol/l per min)				
Placebo	292 (223; 316)	260 (213; 288)	-16 (-55; 71)	
Prednisolone	278 (256; 364)	334 (320; 391)	44 (24; 77)	0.05
IGI				
Placebo	127 (117; 160)	115 (87; 182)	-20 (-38; 47)	
Prednisolone	139 (78-163)	155 (79; 161)	22 (-24; 32)	NS
$AUC_{ISR}/AUC_{gluc} \times 1000$				
Placebo	34 (28; 43)	31 (25; 37)	-1 (-8; 6)	
Prednisolone	34 (30; 38)	31 (30; 38)	-2 (-5; 3)	NS

Values are median (interquartile range). Between group changes from baseline are tested using the Mann-Whitney test.

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 standardised meal tests, a 15-day treatment with prednisolone increased AUC_{gluc} ($P<0.001$), despite augmented C-peptide secretion ($P=0.05$; Fig. 3, panels A–D and Table 2).

Parameters of β -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_{ISR}/AUC_{gluc} ratio, glucose sensitivity and rate sensitivity (Fig. 4, panels A–D and Table 2). In multiple regression analysis, the PFR and OGIS similarly affected the change in AUC_{gluc} during the meal tests (standardised correlation coefficients -0.482 and -0.500 for PFR and OGIS respectively).

Comparisons between subjects of both protocols

No statistical significant differences were measured between the subjects of the acute and 2-week protocol, regarding age, BMI, physical activity and metabolic parameters at baseline.

Experience of prednisolone-related symptoms

Subjects treated with prednisolone did not report increased physical complaints compared to

placebo-treated subjects. The completion rate of both studies was 100%; none of the participants withdrew due to side effects of prednisolone exposure.

Discussion

GCs are well known to perturb glucose metabolism in humans, which most often is attributed to reduction in insulin sensitivity. It is far less clear to what extent β -cell dysfunction contributes to the diabetogenic effects of GCs. In this study, we demonstrate that prednisolone, as the most widely prescribed oral GC worldwide, in addition to reducing insulin sensitivity, impairs β -cell function in healthy men, both following acute and 2-week exposure. In contrast to previous studies, we assessed the effects of GCs on β -cell function under daily life conditions, i.e. during standardised meal tests (19).

A single dose of 75 mg of prednisolone acutely increased AUC_{gluc}, while C-peptide secretion failed to respond (Fig. 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 β -cells to hyperglycaemia. In line with these observations, Plat *et al.* demonstrated that elevation of morning cortisol levels acutely impairs insulin secretion (25). In our study, all measured β -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

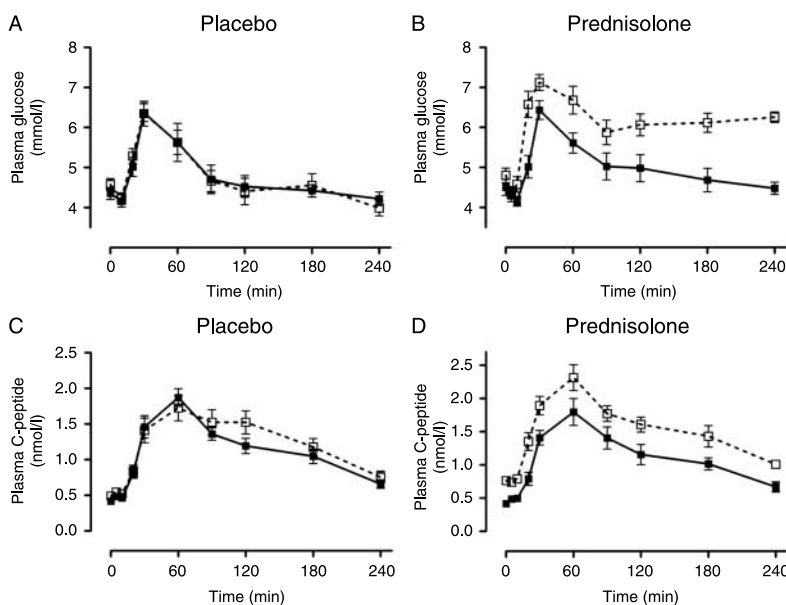


Figure 3 A 2-week treatment with prednisolone increased area under the postprandial glucose curve (panels A + B), despite increased C-peptide levels (panels C + D). Solid line with black squares represents day 0, and dotted line with white squares represents day 15. Data are mean \pm S.E.M.

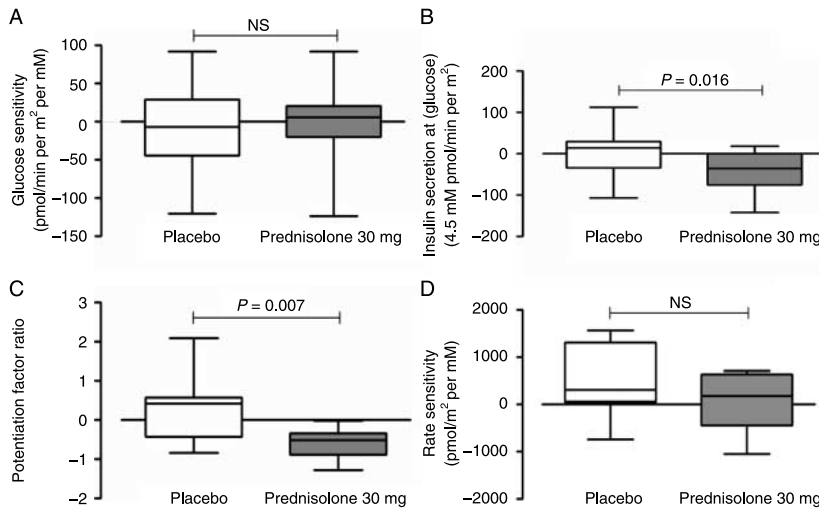


Figure 4 A 15-day treatment with prednisolone decreased the basal secretory tone and potentiation factor ratio (panels B and C). Glucose sensitivity and rate sensitivity were not affected (panels A and D). Graphs are Box and Whisker plots with median and interquartile range.

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, β -cell function appeared to have recovered from the acute effects of prednisolone, since fasting insulin secretion and insulin secretion during the standardised meal test were increased. This may indicate delayed compensation for the IR 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 30 mg of prednisolone daily for 15 days increased C-peptide secretion during prednisolone treatment (Fig. 3). Despite this enhanced secretion, fasting glucose and postprandial AUC_{gluc} exceeded baseline levels, indicating a relative hypoinsulinaemia and accordingly a decline in β -cell function. This relative hypoinsulinaemia under fasting conditions becomes evident in our β -cell model parameter 'insulin secretory tone at a glucose level of 4.5 mM', in which ISRs 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 2-week prednisolone treatment.

Previous studies in which subjects were exposed for multiple days to GCs also reported increased insulin secretion, as assessed by hyperglycaemic clamps (8, 9, 11, 12) and IVGTTs (10). 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 IR 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 IR (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 β -cell function. Our experimental design may be more physiological compared to tests using i.v. glucose, since the former comprises the contribution of multiple factors, including incretins (17), non-glucose metabolic stimuli, such as non-esterified fatty acids (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 β -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 β -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. Additional investigation is required to identify the specific non-glucose stimuli for insulin secretion that are impaired by GC treatment.

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 2-week study. We propose that GCs induce an acute inhibitory effect on β -cell function, as extensively demonstrated in both *in vitro* and *in vivo* experiments, but that β -cell function partly recovers following more prolonged exposure. In the latter situation, GC-induced IR may oppose the direct effect of GCs on the β -cell by enhancing insulin secretion. However, it should 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 β -cell function, both following acute and short-term treatment, in healthy, normoglycaemic men, when measured under physiological, daily life conditions. These data indicate that GC-induced β -cell dysfunction, in addition to IR, contributes to the development of steroid diabetes.

Declaration of interest

D van Raalte, V Nofrate, M C Bunck, T van Iersel, A Mari and M Diamant have nothing to declare. R J Heine is employed by and owns stocks of Eli Lilly & Company. J E Schaap, U K Nässander and W M Dokter are employees of Schering-Plough Research Institute.

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