Clinical Endocrinology (2012) **77**, 224–232 doi: 10.1111/j.1365-2265.2011.04242.x

ORIGINAL ARTICLE

Twenty-four-hour profiles of plasma glucose, insulin, C-peptide and free fatty acid in subjects with varying degrees of glucose tolerance following short-term, medium-dose prednisone (20 mg/day) treatment: evidence for differing effects on insulin secretion and action

Kevin C. J. Yuen, Patricia A. McDaniel and Matthew C. Riddle

Division of Endocrinology, Diabetes and Clinical Nutrition, Department of Medicine, Oregon Health and Science University, Portland, OR, USA

Summary

Objective To determine the time course and prandial effects of short-term, medium-dose prednisone on 24-h metabolic patterns under standardized conditions.

Context Glucocorticoids (GCs) adversely affect glucose homoeostasis but 24-h profiles of glucose, insulin, C-peptide and free fatty acids (FFAs) following short-term, medium-dose prednisone treatment in persons with varying degrees of glucose tolerance are not well defined.

Design An open-label cross-sectional interventional study.

Subjects Three groups were prospectively studied: persons with type 2 diabetes (T2DM; $n = 7$), persons 'at risk' for T2DM (AR; $n = 8$) and persons with normal glucose tolerance (NGT; $n = 5$).

Methods Before and after 3-day treatment with prednisone 20 mg each morning, subjects underwent 24-h frequent blood sampling. Eucaloric mixed meals were provided at 08:00, 12:00 and 18:00 h. Insulin/glucose ratio provided an estimate of β-cell response to meal stimuli.

Measurements Plasma glucose, insulin, C-peptide, haemoglobin A1c and FFA.

Results Prednisone induced greater increases in glucose levels from midday ($P = 0{\cdot}001$) to midnight ($P = 0{\cdot}02$) in the T2DM than the AR and NGT groups. In contrast, insulin ($P = 0{\cdot}03$) and C-peptide ($P = 0{\cdot}04$) levels decreased postbreakfast in the T2DM group, whereas no changes in the morning but higher C-peptide levels ($P = 0{\cdot}03$) from midday to midnight were observed in the AR group. In the T2DM group, insulin/glucose ratio decreased postbreakfast ($P = 0{\cdot}04$) and increased postdinner ($P = 0{\cdot}03$). Fasting glucose, insulin and C-peptide levels were unchanged in all groups, and FFA levels modestly increased postdinner ($P = 0{\cdot}03$) in the NGT group.

Conclusion Short-term, medium-dose prednisone treatment induces postprandial hyperglycaemia in T2DM and AR predominantly from midday to midnight because of suppression of insulin secretion followed by decreased insulin action that dissipates overnight. Effective treatment of prednisone-induced hyperglycaemia should target both rapid onset relative insulin deficiency and a less than 24-h total duration of effect.

(Received 12 July 2011; returned for revision 28 July 2011; finally revised 26 September 2011; accepted 26 September 2011)

Correspondence: Kevin C. J. Yuen, MRCP(UK), MD, Division of Endocrinology, Diabetes and Clinical Nutrition, Department of Medicine, Oregon Health and Science University, 3181 SW Sam Jackson Park Road, Mailcode: L607, Portland, OR 97239-3098, USA. Tel.: +1 503 494 0175; Fax: +1 503 494 6990; E-mail: yuenk@ohsu.edu

Introduction

Oral glucocorticoids (GCs) are commonly prescribed in clinical practice to treat a broad range of acute and chronic inflammatory and autoimmune diseases.[1] Although GCs are usually well tolerated, especially if used briefly, their usage is associated with a significant number of metabolic adverse effects, including insulin resistance, visceral adiposity, dyslipidaemia and fasting and postprandial hyperglycaemia.[2] Prominent hyperglycaemia is most often observed when high dosages of GC are taken by persons with known diabetes, but may also occur at moderate and low doses in persons not previously known to be at risk.[3,4] This unwanted hyperglycaemic effect is generally attributed to the reduction in sensitivity to insulin action in muscle, liver and adipose tissue.[5–7] In addition, animal studies[8,9] have demonstrated direct inhibitory effects of GCs on insulin secretion and adverse effects on β-cell function in some human studies.[10,11]

However, previous studies have generally used high doses of long-acting GCs such as dexamethasone or betamethasone that are infrequently utilized for ambulatory treatment in clinical practice, and β-cell function has been assessed by tests using intravenous glucose loads such as the intravenous glucose tolerance test or the

hyperglycaemic clamp[12–14] rather than standardized meals. Furthermore, published data on the mechanisms and time-course action of medium-dose prednisone, the GC that is most often used in clinical practice,[1] are limited.

Therefore, to determine the time course and prandial effects of short-term, medium-dose prednisone under standardized conditions in subjects with varying degrees of glucose tolerance, we examined 24-h profiles of glucose, insulin, C-peptide and free fatty acids (FFAs) in groups of subjects with well-controlled type 2 diabetes (T2DM), 'at risk' (AR) subjects and healthy subjects known to have normal glucose tolerance (NGT) before and after 3-day treatment with 20 mg of prednisone administered orally in the morning.

Patients and methods

Study participants

Subjects with well-controlled T2DM ($n = 7$) and AR ($n = 8$) subjects [individuals with impaired glucose tolerance ($n = 2$) or prior gestational diabetes and first-degree relatives with T2DM ($n = 6$)] were recruited from the Diabetes Center at Oregon Health and Science University, Portland, Oregon, whereas healthy volunteers with NGT ($n = 5$) were recruited via local advertisements. Subjects with T2DM were diagnosed according to the criteria set forth by the American Diabetes Association[15] and had haemoglobin A1c levels ranging between 6·8% and 7·8%. All subjects with T2DM were not on any oral antidiabetic medications and were treated with diet alone. Subjects were deemed to have impaired glucose tolerance if they had fasting plasma glucose levels ranging between 5·5 and 7·0 mM and 120-min plasma glucose levels ranging between 7·8 and 11·0 mM following a 75 g oral glucose tolerance test. Subjects with NGT were enrolled if they met the following criteria: age 25–65 years, no family history of diabetes, normal 75 g oral glucose tolerance test within 3 months of study entry, not on any medications and a nonpregnant state for female subjects confirmed by a pregnancy test. Female subjects were either not on any oestrogen replacement or were on a stable dose of oestrogen replacement for at least 6 months prior to study entry. All subjects were asked to maintain their normal physical activity and diet for at least 3 days before the investigations and were free from any illnesses at the time of study entry. The study was approved by the Oregon Health and Science University Institutional Review Board, and all subjects provided written informed consent before taking part in the study.

Study design

Subjects underwent a 75 g oral glucose tolerance test within 3 months before study entry and were not taking any medications known to affect carbohydrate metabolism (Fig. 1). Haemoglobin A1c levels were measured in all subjects at a screening visit 2–4 weeks before study entry. Eligible subjects were admitted to the General Clinical Research Center (GCRC) at Oregon Health and Science University for two impatient visits (Visits 1 and 2). At each GCRC visit, all subjects were admitted at 21:00 h, where history and physical examination were performed, and weight and height were measured. From 22:00 h onwards, all subjects began fasting and only sips of water were permitted overnight. An intravenous cannula was placed in the antecubital fossa at 08:00 h the following day for frequent blood sampling at 08:00, 09:00, 10:00, 12:00, 13:00, 14:00, 16:00, 18:00, 19:00, 20:00, 22:00, 00:00, 03:00, 06:00, 07:00 and 08:00 h for measurement of plasma glucose, insulin, C-peptide and FFA levels. All subjects were given a diet of 35 kcal/kg/day with a caloric distribution of 20% breakfast, 30% lunch and 50% dinner, based upon a typical daily caloric distribution that is reflective in the United States population. All meals were served at 08:00, 12:00 and 18:00 h, and no snacks were permitted between those times. The breakdown of the meals was kept stable at 50% carbohydrate, 30% fat and 20% protein. Subjects were instructed to eat all the food that was served. Following the completion of Visit 1, all subjects were instructed to take 20 mg of prednisone orally for 3 consecutive days (Days 1–3) at 08:00 h. Subjects were re-admitted to the GCRC for Visit 2 at 22:00 h on Day 2. After taking 20 mg of prednisone orally at 08:00 h on Day 3, the subjects then underwent a second 24-h frequent blood sampling phase before being discharged from the GCRC the following day.

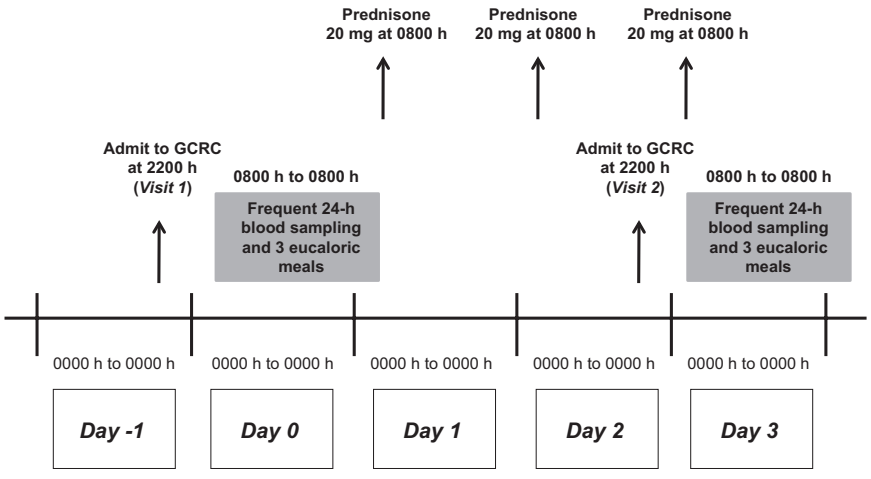

Fig. 1 Study design.

Assays

Plasma glucose concentrations were assayed by a glucose oxidase and peroxidase enzymatic method (Glucose enzymatique PAP 7500; BioMérieux SA, Marcy-L'étoile, France) using a Beckman analyzer (Beckman Glucose Analyzer; Beckman, Fullerton, CA, USA). The coefficient of variation of this reference method was <3%. Plasma insulin and C-peptide concentrations were measured with a radioimmunoassay method, which has little or no proinsulin or C-peptide cross-reactivity and has a reported average coefficient of variation of <10% (Linco Research, St. Charles, MO, USA).[16] Plasma FFA concentrations were measured with an enzymatic calorimetric method using the WAKO kit (HR Series NEFA; Wako Chemicals USA, Richmond, VA, USA) with an intra-assay variation of 1%, inter-assay variation of 4–15% and a detection limit of 0·02 mM. We measured red blood cell haemoglobin A1c by a temperature controlled high- performance liquid chromatography method; against standards of 5·6% and 9·6%, the coefficients of variation for haemoglobin A1c were <2·5% (Boehringer Mannheim, Germany).[17–19]

Calculations

The insulin/glucose ratio was calculated by dividing insulin by glucose at each time point. Using the trapezoidal rule, the suprabasal incremental area under the curve (AUC) estimated the AUCs for glucose, insulin, C-peptide, FFAs and the insulin/glucose ratio at 4 time blocks during the 24-h period: 08:00–12:00, 12:00–18:00, 18:00–00:00 and 00:00–08:00 h.

Statistical analysis

The primary endpoint was change in AUCs of glucose and insulin over intervals following three eucaloric meals before and after 3 days treatment with prednisone. Secondary endpoints included change in β-cell responses to meals and the effect of prednisone treatment on FFA levels. All data are presented as mean ± SEM. Distributions of residuals were examined for normality by graphical methods. Within-group before *vs* after prednisone treatment comparisons were performed by paired Student's *t*-test and Wilcoxon signed-rank test for non-normally distributed data. Between-group baseline characteristics were tested using one-way ANOVAs, whereas the differential effects of prednisone treatment between groups (T2DM *vs* AR *vs* NGT) on the incremental AUCs were tested using two-way ANOVA. *Post hoc* tests were undertaken using the Dunn or the Bonferroni test for nonparametric and parametric data, respectively. A *P*-value of ≤0·05 was deemed statistically significant in all tests.

Results

Baseline characteristics

The subjects in the three groups were well matched with regard to weight and BMI measurements (Table 1). The subjects in the AR group had either impaired fasting glucose levels or impaired glucose tolerance or both and were younger than the subjects with T2DM ($P = 0·005$) and NGT ($P = 0·04$). The haemoglobin A1c levels were significantly higher in the T2DM group compared with the AR ($P = 0·03$) and NGT ($P = 0·01$) groups. All enrolled subjects completed the study.

Fasting measurements

No significant changes were observed for fasting glucose, FFA, insulin, C-peptide and insulin/glucose ratio before and after prednisone treatment for the three groups of subjects (Table 1).

24-h profiles of glucose, FFA, insulin and C-peptide

Prednisone treatment increased glucose levels in the T2DM and AR groups from 12:00 to 00:00 h and from 12:00 to 20:00 h, respectively (Figs 2 and 3). In the NGT group, glucose levels were increased at 12:00, 13:00 and 18:00 h. Prednisone treatment

Table 1. Baseline characteristics and fasting biochemical parameters before and after prednisone treatment

	T2DM ($n = 7$)		AR ($n = 8$)		NGT ($n = 5$)	
Gender (males/females)	4/3		2/6		3/2	
Age (years)	**58·0 ± 3·8**		**39·1 ± 3·5***		**50·2 ± 4·5**	
Weight (kg)	92·0 ± 7·9		81·5 ± 10·3		90·0 ± 5·0	
BMI (kg/m^2)	32·7 ± 2·7		29·9 ± 2·8		31·7 ± 1·6	
Haemoglobin A1c (%)	**7·2 ± 0·1†**		**6·0 ± 0·1**		**5·2 ± 0·1**	
	Before	After	Before	After	Before	After
Fasting glucose (mM)	6·4 ± 0·6	6·2 ± 0·5	5·1 ± 0·2	5·2 ± 0·3	4·7 ± 0·1	4·6 ± 0·1
Fasting insulin (pM)	102 ± 35	103 ± 25	97 ± 14	81 ± 13	84 ± 13	70 ± 12
Fasting C-peptide (nM)	758 ± 268	693 ± 250	663 ± 250	594 ± 95	470 ± 99	297 ± 34
Fasting FFA (mM)	0·74 ± 0·11	0·68 ± 0·15	0·50 ± 0·03	0·60 ± 0·06	0·52 ± 0·11	0·50 ± 0·08
Insulin/glucose ratio (ins/gluc)	14·9 ± 3·4	16·3 ± 3·0	19·0 ± 2·4	15·3 ± 2·4	18·0 ± 3·0	15·3 ± 2·5

NGT, subjects with normal glucose tolerance; AR, subjects 'at risk' of developing diabetes; T2DM, subjects with type 2 diabetes; FFA, free fatty acid. Bold values indicate between-group statistical significance.
*ANOVA, $P = 0·04$ and †ANOVA, $P = 0·01$.

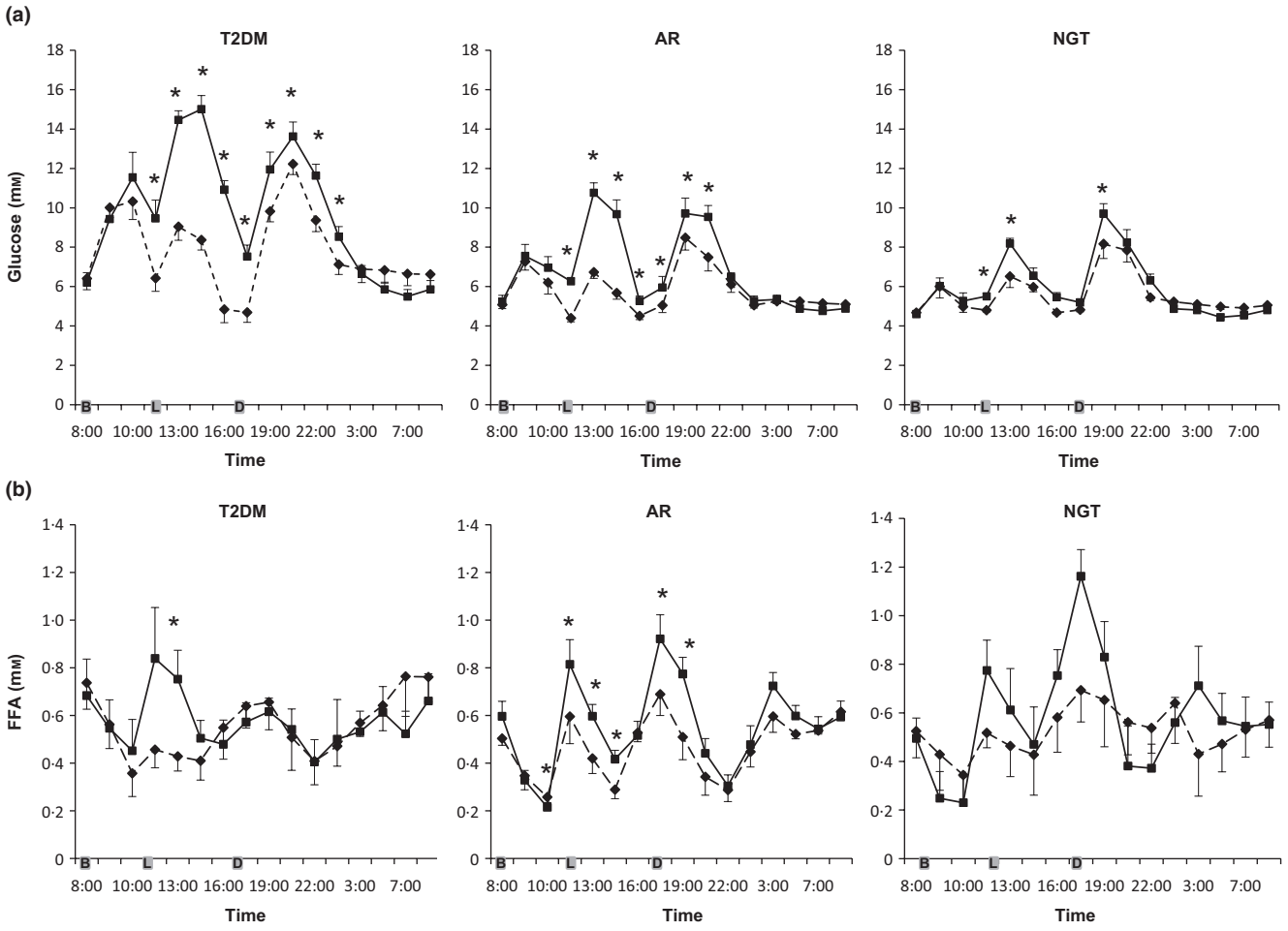

Fig. 2 Twenty-four-hour time profile changes for (a) glucose and (b) FFAs. T2DM: subjects with type 2 diabetes, AR: subjects 'at risk' of developing diabetes, normal glucose tolerance (NGT): subjects with NGT, B: breakfast, L: lunch and D: dinner. ♦ and dotted line represent before prednisone treatment, and ■ and solid line represent after prednisone treatment. Data are presented as mean ± SEM. The *x*-axis signifies time of day in military time. *$P < 0.05$ for within-group comparisons between before and after prednisone treatment at each time point.

increased FFA levels in the T2DM group at 13:00 h, in the AR group from 10:00 to 14:00 h and at 18:00 to 19:00 h, whereas in the NGT group, prednisone treatment did not alter FFA levels at any time point. Prednisone treatment decreased insulin levels in the T2DM group at 09:00, 10:00 and 13:00 h, but increased these levels at 16:00 and 18:00 h. In contrast, in the AR and NGT groups, prednisone treatment increased insulin levels at 14:00, 16:00, 19:00 and 20:00 h, and at 18:00, 20:00 and 22:00 h, respectively. For C-peptide levels, prednisone treatment decreased C-peptide levels in the T2DM group from 10:00 to 14:00 h, whereas in AR and NGT groups C-peptide levels were unchanged.

Incremental AUCs of glucose, FFA, insulin and C-peptide

Prednisone treatment significantly increased the incremental AUC_{gluc} postbreakfast, postlunch and postdinner in the T2DM and AR groups, and postlunch and postdinner in the NGT group (Tables 2 and 3). In addition, the between-group incremental AUC_{gluc} postlunch and postdinner were significantly different (ANOVA, $P = 0{\cdot}001$ and $P = 0{\cdot}02$, respectively) with the greatest increment observed in the T2DM group. However, by 00:00 h, glucose levels had returned to pretreatment levels in all three groups.

Prednisone treatment did not alter the incremental AUC_{FFA} postbreakfast, postlunch and postdinner in the T2DM and AR groups, and increased the incremental AUC_{FFA} postdinner in the NGT group. However, between-group comparison of the incremental AUC_{FFA} was not significantly different, and by 00:00 h, FFA levels had returned to pretreatment levels in all three groups.

In contrast, prednisone treatment decreased the incremental AUC_{ins} postbreakfast but did not alter the incremental AUC_{ins} postlunch and postdinner in the T2DM group, and increased the incremental AUC_{ins} postlunch and postdinner in the AR and NGT groups. When compared between groups, the incremental AUC_{ins} postbreakfast was significantly lower in the T2DM compared with the AR and NGT groups (ANOVA, $P = 0{\cdot}03$), but no between-group differences were observed for the incremental AUC_{ins} postlunch and postdinner. By 00:00 h, insulin levels had all returned to baseline levels in all three groups.

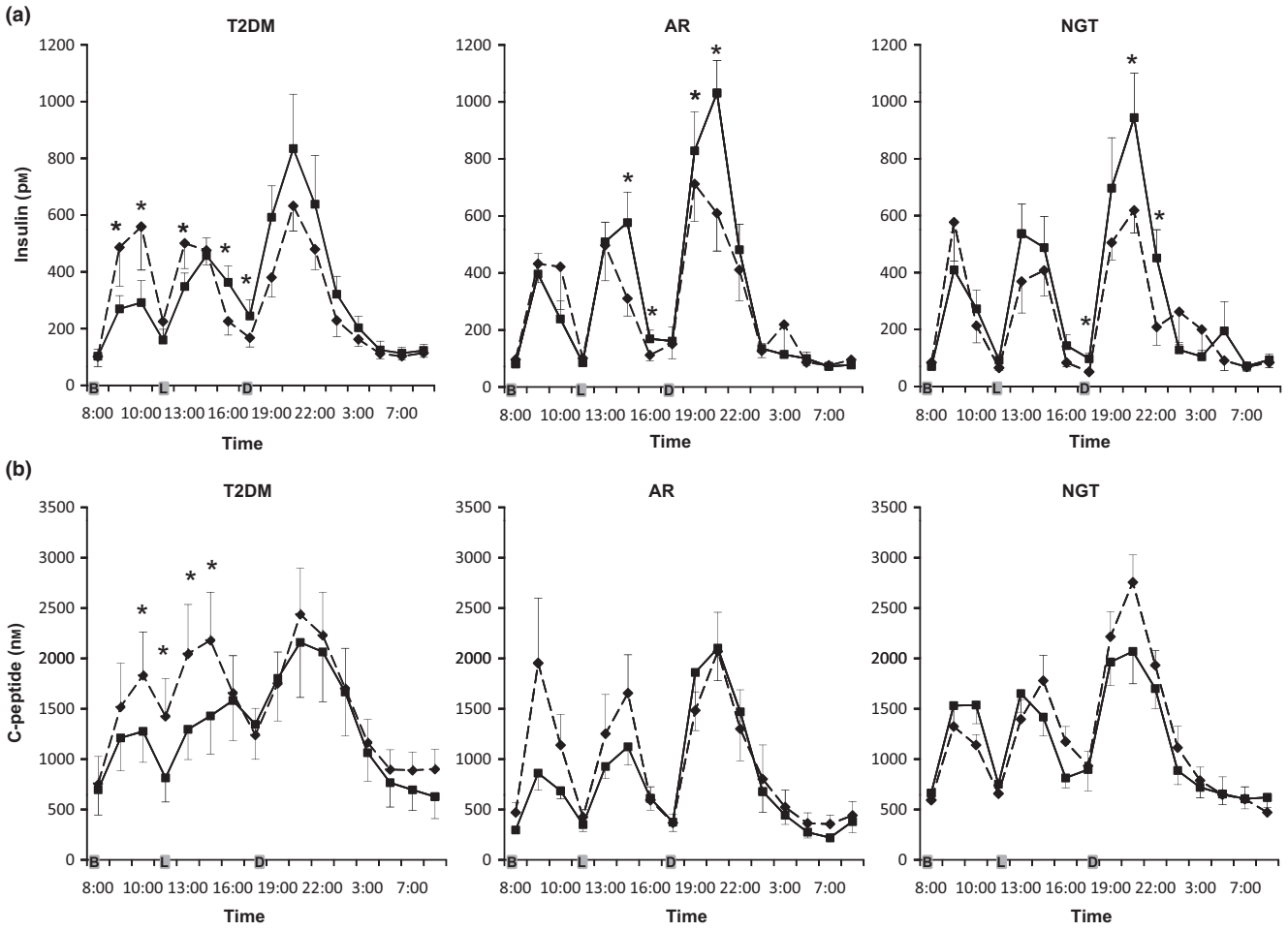

Fig. 3 Twenty-four-hour time profile changes for (a) insulin and (b) C-peptide levels. T2DM: subjects with type 2 diabetes, AR: subjects 'at risk' of developing diabetes, normal glucose tolerance (NGT): subjects with NGT, B: breakfast, L: lunch and D: dinner. ♦ and dotted line represent before prednisone treatment and ■ and solid line represent after prednisone treatment. Data are presented as mean ± SEM. The *x*-axis signifies time of day in military time.
*$P < 0.05$ for within-group comparisons between before and after prednisone treatment at each time point.

Finally, prednisone treatment decreased the incremental $AUC_{C\text{-}pep}$ postbreakfast in the T2DM group and increased the incremental $AUC_{C\text{-}pep}$ postlunch and postdinner in the AR group, but did not alter the incremental $AUC_{C\text{-}pep}$ in the NGT group. When compared between groups, the incremental $AUC_{C\text{-}pep}$ postbreakfast was significantly lower in the T2DM compared with the AR and NGT groups (ANOVA, $P = 0{\cdot}02$), but no between-group differences were observed for the incremental $AUC_{C\text{-}pep}$ postlunch and postdinner. By 00:00 h, insulin levels had all returned to baseline levels.

24-h profile and incremental AUCs of the insulin/glucose ratio

In the T2DM group, prednisone treatment decreased the insulin/glucose ratio at 10:00 and 12:00 h and increased the insulin/glucose ratio at 19:00 and 20:00 h, whereas in the AR group, the insulin/glucose ratio decreased at 10:00 h and increased at 16:00 and 20:00 h (Fig. 4, Tables 2 and 3). This contrasts with the NGT group where no changes were observed postbreakfast and postlunch, but was increased at 18:00, 20:00 and 22:00 h.

Additionally, the incremental AUC of insulin/glucose ratio (AUC_{IG}) decreased by 77% postbreakfast ($P = 0{\cdot}04$) and increased by 77% postdinner ($P = 0{\cdot}03$) in the T2DM group, decreased by 44% postbreakfast ($P = 0{\cdot}05$) in the AR group, and increased by 66% postlunch ($P = 0{\cdot}03$) and by 59% postdinner ($P = 0{\cdot}03$) in the NGT group. The between-group incremental AUC_{IG} postlunch was significantly different (ANOVA, $P = 0{\cdot}01$), but not for postbreakfast and postdinner. By 00:00 h, the insulin/glucose ratio had returned to pretreatment levels in all three groups.

Discussion

This study provides the first detailed analysis of the 24-h time-course pattern and prandial effects of hyperglycaemia induced by a GC commonly used in clinical practice and administered at a medium dose in subjects with varying degrees of glucose tolerance. Using a relatively short exposure time of 3 days, we found that medium-dose prednisone treatment induced pronounced daytime elevations in glucose levels in the T2DM group, milder elevations in the AR group and minimal effects in the NGT group. The time

Table 2. Biochemical parameters before and after 3 days of prednisone treatment

	08:00–12:00 h (postbreakfast)		12:00–18:00 h (postlunch)		18:00–00:00 h (postdinner)		00:00–08:00 h (overnight)	
Time	Before	After	Before	After	Before	After	Before	After
AUC_{gluc} (mM/h)								
T2DM	**9·5 ± 1·7**	**14·5 ± 1·9***	**0·7 ± 2·1**	**33·8 ± 2·5†**	**17·9 ± 3·1**	**30·7 ± 3·8†**	3·7 ± 2·5	3·2 ± 1·8
AR	**3·2 ± 0·9**	**6·0 ± 0·8†**	**1·0 ± 0·9**	**13·5 ± 2·1†**	**9·0 ± 1·6**	**13·8 ± 1·6***	0·9 ± 0·9	−0·9 ± 3·0
NGT	1·9 ± 0·6	3·3 ± 0·8	**4·0 ± 0·7**	**9·2 ± 0·8***	**10·4 ± 1·9**	**14·5 ± 2·2†**	3·1 ± 0·9	0·7 ± 0·7
ANOVA, *P* between-groups		NS		0·001		0·02		NS
AUC_{FFA} (mM/h)								
T2DM	−1·02 ± 0·25	−0·33 ± 0·45	−1·41 ± 0·41	−0·64 ± 0·69	−1·40 ± 0·57	−1·08 ± 0·88	−1·05 ± 0·53	−1·04 ± 0·98
AR	−0·43 ± 0·14	−0·62 ± 0·25	−0·13 ± 0·15	0 ± 0·52	−0·63 ± 0·30	−0·59 ± 0·55	0·32 ± 0·30	0·15 ± 0·56
NGT	−0·52 ± 0·27	−0·36 ± 0·18	0·76 ± 0·42	1·40 ± 0·32	**−0·24 ± 0·50**	**0·32 ± 0·36***	0·52 ± 0·59	0·97 ± 0·48
ANOVA, *P* between-groups		NS		NS		NS		NS
AUC_{ins} (pM/h)								
T2DM	**1194 ± 382**	**507 ± 142***	1335 ± 166	1469 ± 188	1990 ± 314	2948 ± 729	399 ± 221	693 ± 132
AR	828 ± 255	557 ± 101	**808 ± 175**	**1431 ± 218†**	**2072 ± 427**	**3070 ± 366***	372 ± 326	208 ± 119
NGT	668 ± 156	664 ± 211	**727 ± 186**	**1276 ± 266***	**1634 ± 221**	**2770 ± 440***	614 ± 516	453 ± 243
ANOVA, *P* between-groups		0·03		NS		NS		NS
$AUC_{C\text{-}peptide}$ (nM/h)								
T2DM	**3039 ± 539**	**1502 ± 541***	6033 ± 1179	4179 ± 1206	7642 ± 1569	7331 ± 1832	3117 ± 1309	2663 ± 968
AR	2268 ± 383	1614 ± 182	**2698 ± 582**	**4112 ± 425***	**5826 ± 912**	**8233 ± 577***	412 ± 410	1424 ± 674
NGT	2441 ± 823	1201 ± 207	2681 ± 743	2602 ± 513	5352 ± 1310	7038 ± 1938	315 ± 746	933 ± 628
ANOVA, *P* between-groups		0·02		NS		NS		NS
AUC_{IG}								
T2DM	**120 ± 49**	**28 ± 16***	199 ± 31	129 ± 22	**182 ± 41**	**322 ± 51***	55 ± 28	41 ± 35
AR	**105 ± 26**	**59 ± 9***	126 ± 29	135 ± 18	252 ± 49	592 ± 176	66 ± 62	81 ± 34
NGT	117 ± 37	104 ± 32	**102 ± 25**	**169 ± 41***	**207 ± 29**	**329 ± 46***	116 ± 109	92 ± 47
ANOVA, *P* between-groups		NS		0·01		NS		NS

AUC, area under the curve; NGT, subjects with normal glucose tolerance; AR, subjects 'at risk' of developing diabetes; T2DM, subjects with type 2 diabetes; NS, not significant; Δ, change.
Bold values indicate within-group statistical significance (paired *t*-test): $^*P < 0{\cdot}05$ and $\dagger P < 0{\cdot}01$ *vs* before treatment.

course of glucose changes in both the T2DM and AR groups showed greatest effects by midday but then dissipated by midnight. Our data are in line with those recently reported by Burt *et al.*,[20] but further extend their findings by demonstrating that the changes in the patterns of insulin and C-peptide levels strongly suggest early suppression of insulin secretion in the T2DM group and later onset of decreased insulin action in all three groups, followed by complete resolution of all metabolic parameters by the next morning.

Despite the recognition that GCs predominantly increase postprandial blood glucose,[21] there is limited published evidence for this pattern of metabolic excursions. Thus, our results provide clinically relevant insights. Decreased levels of plasma insulin, C-peptide and the insulin/glucose ratio were already apparent in the T2DM group between 09:00 and 10:00 h (i.e. 1–2 h after taking prednisone and breakfast), implying that a rapid effect on insulin secretion precedes by several hours the onset of relative insensitivity to insulin at target organs to which GC-induced hyperglycaemia is usually ascribed. GCs have been previously shown to induce peripheral hyperinsulinemia because of prehepatic β-cell insulin hypersecretion that is partially ameliorated by a concomitant increase of hepatic insulin clearance,[22,23] but not with C-peptide as GCs do not affect hepatic extraction of C-peptide[24], and the clearance kinetics of C-peptide is linear.[25] In addition, the rapid suppression of insulin secretion does not appear to persist beyond lunch, by which time the hyperglycaemic effect because of insensitivity of tissues becomes more apparent in both the T2DM and AR groups. The higher levels of insulin and the greater magnitude of the insulin/glucose ratio later in the day suggest of β-cell compensation for the delayed insulin resistance at that time. This pattern is consistent with evidence that other biologic effects of similar doses of orally administered prednisone reach a peak between 4 and 8 h and have a duration of action lasting in the range of 12–16 h.[26] Intuitively, the choice of insulin to control daytime prednisone-induced hyperglycaemia should target not only glucose increases at midday and evening, but the first few hours after breakfast and to a lesser degree after midnight. For example, a morning injection of premixed or patient-mixed NPH with a rapid-acting insulin analog or regular insulin followed by an injection of rapid-acting or regular insulin with the evening meal might be preferable to use of NPH or a long-acting insulin analog alone in subjects with T2DM or impaired glucose tolerance.

The mechanisms by which β-cell secretion may be altered by prednisone and other GCs are not well understood. Glucocorticoid-induced hyperinsulinaemia is regarded as an adaptation of pancreatic β-cell function to the GC-induced insulin resistance in

Table 3. Schematic representation of the time-dependent within-group changes of the biochemical parameters

Time	08:00–12:00 h	12:00–18:00 h	18:00–00:00 h	00:00–08:00 h
Glucose (mM)				
T2DM	↑	↑	↑	↔
AR	↑	↑	↑	↔
NGT	↔	↑	↑	↔
FFA (mM)				
T2DM	↔	↔	↔	↔
AR	↔	↔	↔	↔
NGT	↔	↔	↑	↑
Insulin (pM)				
T2DM	↓	↔	↔	↔
AR	↔	↑	↑	↔
NGT	↔	↑	↑	↔
C-peptide (nM)				
T2DM	↓	↔	↔	↔
AR	↔	↑	↑	↔
NGT	↔	↔	↔	↔
Insulin/glucose ratio				
T2DM	↓	↔	↑	↔
AR	↓	↔	↔	↔
NGT	↔	↑	↑	↔

NGT, subjects with normal glucose tolerance; AR, subjects 'at risk' of developing diabetes; T2DM, subjects with type 2 diabetes; ↑, increased; ↓, decreased; and ↔, no change compared to before treatment.

liver, muscle and adipose tissues, either through hyperglycaemia, by other signalling mechanisms, or most likely a combination of these mechanisms.[27,28] As GC receptors are known to be present on β-cells,[29,30] it is possible that GCs may also exert direct stimulatory and inhibitory effects on insulin secretion. In a recent study by van Raalte *et al.*[11] in healthy men, acute treatment with 75 mg/day of prednisolone resulted in an acute inhibitory effect of the β-cell, but longer exposure with a prednisolone dose of 30 mg/day resulted in fasting and postprandial hyperglycaemia but relative hypoinsulinemia, which was thought to be secondary to the insufficient compensatory β-cell response. This contrasts with our findings where our subjects with NGT did not demonstrate any tendency towards prednisone-induced suppression of insulin secretion, suggesting that the ability to compensate for the decrease in insulin action with corresponding increases in insulin secretion may be related to the severity of the underlying β-cell defect and higher doses of prednisolone used. Whether our findings can be generalized to other forms of GCs is not clear, but it is noteworthy that betamethasone[31] and dexamethasone[32] have been previously shown to induce greater glucose intolerance and insulin resistance than prednisone and deflazacort[31,33] in healthy humans.

In addition, our data do not clarify the site or mechanisms responsible for daytime prednisone-induced insulin resistance (hepatic *vs* muscle or adipose tissue). Although elevation of circulating FFAs resulting from GC-induced lipolysis offer a potential explanation of the mechanism for alteration of either β-cell or target-tissue function,[34] our findings do not provide much support for this possibility. Notably, in this study, we did not observe any changes in circulating levels of FFAs in the T2DM and AR groups after prednisone treatment. Therefore, we believe the following can be surmised from our findings: (i) individuals with an inherent β-cell defect may be susceptible to rapid suppression of insulin secretion by medium-dose prednisone, an effect which contributes significantly to prednisone-induced daytime hyperglycaemia, whereas this phenomenon does not significantly affect subjects with NGT and (ii) prednisone-induced insensitivity to insulin occurs later in the day and may be less well compensated by β-cell adaptation in the T2DM and AR subjects.

We acknowledge that interpretation of the mechanisms underlying prednisone-induced hyperglycaemia in our study is limited by the methods used. Because the main purpose of the study was to assess the 24-h time course of hyperglycaemia, β-cell function and insulin sensitivity were assessed indirectly by determining the 24-h

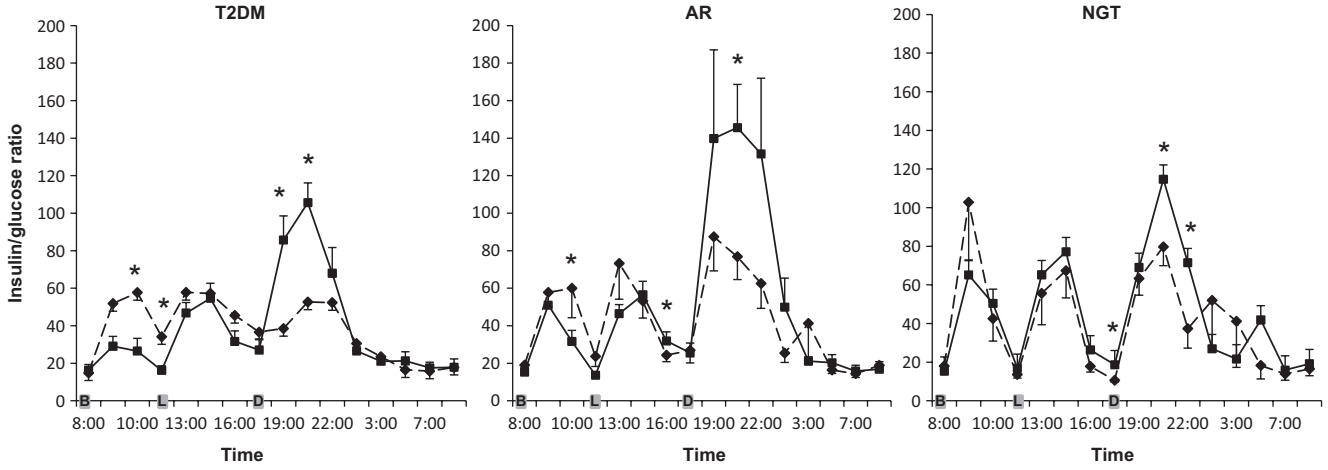

Fig. 4 Twenty-four-hour time profile changes for insulin/glucose ratio. T2DM: subjects with type 2 diabetes, AR: subjects 'at risk' of developing diabetes, normal glucose tolerance (NGT): subjects with NGT, B: breakfast, L: lunch and D: dinner. ♦ and dotted line represent before prednisone treatment and ■ and solid line represent after prednisone treatment. Data are presented as mean ± SEM. The *x*-axis signifies time of day in military time. *$P < 0.05$ for within-group comparisons between before and after prednisone treatment at each time point.

profiles of glucose, insulin, C-peptide and the insulin/glucose ratio. The study design also involved the measurement of variables at 15 time points before and after prednisone; hence, to account for the potential of false positive results with this number of measurements, analyses within-groups and between-groups of the four major time blocks were performed. More accurate assessments of β-cell function and insulin sensitivity to address these mechanisms would require adequately powered studies utilizing glucose clamp methods and more frequent blood sampling to allow deconvolution measurements to estimate insulin secretion rates more accurately. Therefore, our findings call for further studies to confirm the apparent importance of early suppression of insulin secretion by prednisone under these standardized conditions. Other limitations of this study include the unrandomized and cross-sectional study design and the small number of study subjects. As the GC treatment was limited to 20 mg of prednisone administered for only 3 days, our findings cannot be extrapolated to encompass treatment at higher or lower prednisone doses, with other forms of GCs such as bethamethasone or dexamethasone or for longer treatment durations.

In summary, short-term, medium-dose (20 mg/day) morning prednisone treatment under controlled conditions resulted in significant worsening of daytime postprandial hyperglycaemia in the T2DM and AR groups, with minimal effects in the NGT group. Increased hyperglycaemia in subjects with T2DM was transitory, being most notable at midday and dissipated by midnight. Although further studies are needed to directly quantify the relative contributions of suppression of insulin secretion and increased resistance to the effects of insulin action, the pertinent changes in the hormonal patterns observed were consistent with a rapidly appearing yet transitory reduction in endogenous insulin secretion followed by decreased insulin action later that dissipated overnight. Accordingly, treatment of prednisone-induced hyperglycaemia in this setting should take into consideration both a rapid onset of relative insulin deficiency followed by a delayed reduction in insulin action on target organs, with the total duration of these effects lasting less than 24 h.

Acknowledgements

This work was supported by a grant from the GCRC (grant number: 0346) and in part from the Rose Hastings and Russell Standley Memorial Trusts.

Disclosure

The authors have nothing to disclose.

References

1 van Staa, T.P., Leufkens, H.G., Abenhaim, L. *et al.* (2000) Use of oral corticosteroids in the United Kingdom. *Quarterly Journal of Medicine*, **93**, 105–111.
2 Donihi, A.C., Raval, D., Saul, M. *et al.* (2006) Prevalence and predictors of corticosteroid-related hyperglycemia in hospitalized patients. *Endocrine Practice*, **12**, 358–362.
3 Gulliford, M.C., Charlton, J. & Latinovic, R. (2006) Risk of diabetes associated with prescribed glucocorticoids in a large population. *Diabetes Care*, **29**, 2728–2729.
4 van Raalte, D.H., Brands, M., van der Zijl, N.J. *et al.* (2011) Low-dose glucocorticoid treatment affects multiple aspects of intermediary metabolism in healthy humans: a randomised controlled trial. *Diabetologia*, **54**, 2103–2112.
5 Besse, C., Nicod, N. & Tappy, L. (2005) Changes in insulin secretion and glucose metabolism induced by dexamethasone in lean and obese females. *Obesity Research*, **13**, 306–311.
6 Pagano, G., Cavallo-Perin, P., Cassader, M. *et al.* (1983) An in vivo and in vitro study of the mechanism of prednisone-induced insulin resistance in healthy subjects. *Journal of Clinical Investigation*, **72**, 1814–1820.
7 Subramanian, S., DeRosa, M.A., Bernal-Mizrachi, C. *et al.* (2006) PPARalpha activation elevates blood pressure and does not correct glucocorticoid-induced insulin resistance in humans. *American Journal of Physiology Endocrinology Metabolism*, **291**, E1365–E1371.
8 Lambillotte, C., Gilon, P. & Henquin, J.C. (1997) Direct glucocorticoid inhibition of insulin secretion. An in vitro study of dexamethasone effects in mouse islets. *Journal of Clinical Investigation*, **99**, 414–423.
9 Longano, C.A. & Fletcher, H.P. (1983) Insulin release after acute hydrocortisone treatment in mice. *Metabolism*, **32**, 603–608.
10 Kalhan, S.C. & Adam, P.A. (1975) Inhibitory effect of prednisone on insulin secretion in man: model for duplication of blood glucose concentration. *Journal of Clinical Endocrinology and Metabolism*, **41**, 600–610.
11 van Raalte, D.H., Nofrate, V., Bunck, M.C. *et al.* (2010) Acute and 2-week exposure to prednisolone impair different aspects of beta-cell function in healthy men. *European Journal of Endocrinology*, **162**, 729–735.
12 Grill, V., Pigon, J., Hartling, S.G. *et al.* (1990) Effects of dexamethasone on glucose-induced insulin and proinsulin release in low and high insulin responders. *Metabolism*, **39**, 251–258.
13 Larsson, H. & Ahren, B. (1999) Insulin resistant subjects lack islet adaptation to short-term dexamethasone-induced reduction in insulin sensitivity. *Diabetologia*, **42**, 936–943.
14 Wajngot, A., Giacca, A., Grill, V. *et al.* (1992) The diabetogenic effects of glucocorticoids are more pronounced in low- than in high-insulin responders. *Proceedings of the National Academy of Sciences of the United States of America*, **89**, 6035–6039.
15 American Diabetes Association. (2011) Diagnosis and classification of diabetes mellitus. *Diabetes Care*, **34**(Suppl 1), S62–S69.
16 Wiedmeyer, H.M., Polonsky, K.S., Myers, G.L. *et al.* (2007) International comparison of C-peptide measurements. *Clinical Chemistry*, **53**, 784–787.
17 Meyer, K.A., Conigrave, K.M., Chu, N.F. *et al.* (2003) Alcohol consumption patterns and HbA1c, C-peptide and insulin concentrations in men. *Journal of the American College of Nutrition*, **22**, 185–194.
18 Cole, R.A., Soeldner, J.S., Dunn, P.J. *et al.* (1978) A rapid method for the determination of glycosylated hemoglobins using high pressure liquid chromatography. *Metabolism*, **27**, 289–301.
19 Davis, J.E., McDonald, J.M. & Jarett, L. (1978) A high-performance liquid chromatography method for hemoglobin A1c. *Diabetes*, **27**, 102–107.
20 Burt, M.G., Roberts, G.W., Aguilar-Loza, N.R. *et al.* (2011) Continuous monitoring of circadian glycemic patterns in patients

receiving prednisolone for COPD. *Journal of Clinical Endocrinology and Metabolism*, **96**, 1789–1796.
21 Clore, J.N. & Thurby-Hay, L. (2009) Glucocorticoid-induced hyperglycemia. *Endocrine Practice*, **15**, 469–474.
22 Kautzky-Willer, A., Thomaseth, K., Clodi, M. *et al.* (1996) Beta-cell activity and hepatic insulin extraction following dexamethasone administration in healthy subjects. *Metabolism*, **45**, 486–491.
23 Meier, J.J., Veldhuis, J.D. & Butler, P.C. (2005) Pulsatile insulin secretion dictates systemic insulin delivery by regulating hepatic insulin extraction in humans. *Diabetes*, **54**, 1649–1656.
24 Bruno, A., Carucci, P., Cassader, M. *et al.* (1994) Serum glucose, insulin and C-peptide response to oral glucose after intravenous administration of hydrocortisone and methylprednisolone in man. *European Journal of Clinical Pharmacology*, **46**, 411–415.
25 Polonsky, K., Jaspan, J., Pugh, W. *et al.* (1983) Metabolism of C-peptide in the dog. In vivo demonstration of the absence of hepatic extraction. *Journal of Clinical Investigation*, **72**, 1114–1123.
26 Magee, M.H., Blum, R.A., Lates, C.D. *et al.* (2001) Prednisolone pharmacokinetics and pharmacodynamics in relation to sex and race. *Journal of Clinical Pharmacology*, **41**, 1180–1194.
27 Buren, J., Lai, Y.C., Lundgren, M. *et al.* (2008) Insulin action and signalling in fat and muscle from dexamethasone-treated rats. *Archives of Biochemistry and Biophysics*, **474**, 91–101.
28 Nicod, N., Giusti, V., Besse, C. *et al.* (2003) Metabolic adaptations to dexamethasone-induced insulin resistance in healthy volunteers. *Obesity Research*, **11**, 625–631.
29 Fischer, B., Rausch, U., Wollny, P. *et al.* (1990) Immunohistochemical localization of the glucocorticoid receptor in pancreatic beta-cells of the rat. *Endocrinology*, **126**, 2635–2641.
30 Matthes, H., Kaiser, A., Stier, U. *et al.* (1994) Glucocorticoid receptor gene expression in the exocrine and endocrine rat pancreas. *Endocrinology*, **135**, 476–479.
31 Pagano, G., Bruno, A., Cavallo-Perin, P. *et al.* (1989) Glucose intolerance after short-term administration of corticosteroids in healthy subjects. Prednisone, deflazacort, and betamethasone. *Archives of Internal Medicine*, **149**, 1098–1101.
32 Abdelmannan, D., Tahboub, R., Genuth, S. *et al.* (2010) Effect of dexamethasone on oral glucose tolerance in healthy adults. *Endocrine Practice*, **16**, 770–777.
33 Bruno, A., Pagano, G., Benzi, L. *et al.* (1992) Change in glucose metabolism after long-term treatment with deflazacort and betamethasone. *European Journal of Clinical Pharmacology*, **43**, 47–50.
34 van Raalte, D.H., Ouwens, D.M. & Diamant, M. (2009) Novel insights into glucocorticoid-mediated diabetogenic effects: towards expansion of therapeutic options? *European Journal of Clinical Investigation*, **39**, 81–93.