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Glucocorticoid induced insulin resistance impairs basal but not glucose entrained high-frequency insulin pulsatility in humans

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Abstract

Aims/hypothesis. Type II (non-insulin-dependent) diabetes mellitus is characterized by abnormal insulin secretion, which involves a disrupted basal and glucose-entrained insulin pulsatility, and by insulin resistance. The aim of this study was to examine the influence of glucocorticoid-mediated insulin resistance on the regularity of high frequency insulin pulsatility.
Methods. Eight healthy men (means ± SD; age 24.4 ± 0.5 years, BMI 23.2 ± 0.7 kg/m^2) were examined after prednisolone treatment (30 mg/day) or placebo for 6 days in a double-blind, placebo controlled, cross-over study with a 6-week washout period. Blood was collected every minute for 60 min during baseline and glucose-entrainment. Time-series were assessed by spectral and autocorrelation analyses and a first-phase insulin secretion test was carried out.
Results. Prednisolone treatment led to insulin resistance as expected (HOMA-S; prednisolone vs placebo; 1.85 ± 0.26 vs 1.02 ± 0.10; $p < 0.01$) with exaggerated first-phase insulin secretion (3016 ± 468 pmol/l vs 1688 ± 207 pmol/l; $p < 0.01$), suggesting a stable disposition index. During baseline, normalized spectral power of serum insulin concentration time-series was reduced during prednisolone exposure compared with placebo (8.40 ± 0.95 vs 11.79 ± 1.66; $p < 0.05$) indicating a disturbed high-frequency oscillatory insulin release. A similar trend was observed using autocorrelation analysis (0.23 ± 0.04 vs 0.32 ± 0.07; $p = 0.12$). During glucose entrainment no difference in normalized spectral power or in the autocorrelation coefficient between prednisolone and placebo ($p > 0.1$) was observed.
Conclusion/interpretation. Six days of prednisolone treatment resulted in a pertubed high-frequency insulin release in the fasting state whereas the ability of glucose to entrain insulin secretion was preserved. This indicates a mechanism of pertubed glucose-insulin feedback mechanism which causes irregular oscillatory insulin release. [Diabetologia (2002) 45: 49–55]

Keywords Prednisolone, insulin resistance, glucose entrainment, high frequency insulin pulsatility, spectral analysis, autocorrelation, deconvolution.

Insulin resistance and relative insulin deficiency are pronounced elements in the pathogenesis of Type II (non-insulin-dependent) diabetes mellitus [1, 2]. It has long been debated whether insulin resistance or beta-cell dysfunction is the primary defect in Type II diabetes [3–8]. In this context insulin resistance has been established as a strong predictor for the development of Type II diabetes, whereas this seems to apply to a lesser extend to beta-cell dysfunction. This might in part be due to the insensitivity of tests for beta-cell dysfunction [6, 9, 10]. A dynamic relation between insulin resistance and a compensatory increase in beta-cell mass and beta-cell glucose metabolism has been suggested [11]. Insulin secretion is pulsatile and irregular insulin pulsatility has been re-

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Abbreviations: HOMA-S, Homeostasis model assessment of insulin sensitivity.

ported in subjects predisposed to develop Type II diabetes [12, 13]. The notion of a possible influence of insulin sensitivity on high-frequency insulin pulsatility has been addressed before [14] whereby a strong positive relation between insulin pulse interval and insulin action (i.e. the more insulin sensitive the less frequent pulses) was found as well as an inverse one between this interval and central obesity. Another study showed a negative correlation between the frequency of insulin pulses and glucose clearance in patients with Type II diabetes and in healthy subjects [15].

The aim of this study was to investigate the effect of glucocorticoid-mediated insulin resistance on high-frequency insulin pulsatility in healthy humans. We therefore assessed baseline insulin pulsatility, as well as glucose-pulse-entrainment, a recently established method for in vivo pulsatility studies [16]. This method has been shown to enable the detection of beta-cell dysfunction in vitro [17, 18] and in Type II diabetic patients [19, 20].

Subjects and methods

Subjects. Eight healthy men (Caucasians) participated in the study (means ± SD; age, 24.4 ± 0.5 years, BMI, 23.2 ± 0.8 kg/m^2). All participants had not been taking any medication before the study. The protocol was approved by the Ethics Committee of Aarhus, Denmark and the study was carried out in accordance with the Helsinki Declaration. All subjects were on an isocaloric diet and were not allowed to be involved in severe exercise 3 days before each day of the study.

Design. To assess the influence of short-term (6 days) glucocorticoid-mediated insulin resistance on regularity of baseline and glucose-pulse-entrained time-series of serum insulin concentrations, the subjects were examined after treatment with prednisolone (30 mg/day) or placebo for 6 days each in a double-blind, cross-over study with a 6-week washout period. At day 0 the subjects underwent a physical examination including measurement of height and weight to determine BMI. They were instructed to take prednisolone 15 mg b.i.d. together with breakfast and dinner.

At day 7 in the morning, after an overnight fast (~ 10 h), the subjects were admitted to the Clinical Research Unit at the Medical Department M., University Hospital of Aarhus. At 08.00 hours two catheters (Venflon 17 G/45 mm, BOC Ohmeda AB, Helsingborg, Sweden) were placed in the antecubital veins, for sampling and infusion purposes. A 30-min resting period was allowed before the measurements were initiated. The subjects were examined during baseline and glucose-entrainment, each period lasting 60 min. Blood samples were drawn for measurements of fasting plasma glucose, serum insulin, C-peptide and NEFA at the end of the resting period.

Baseline (non-stimulated insulin pulsatility). Blood was collected every min from time point 0 min to 60 min.

Entrainment. High-frequency insulin release can be controlled by punctuated glucose infusions at frequencies between 7 and 12 min without a breakthrough of spontaneous (non-entrained) pulses in healthy humans [16]. By the application of an entrainment protocol we intended to assess possible changes after prednisolone treatment in the ability of the beta cell to respond to minor glucose excursions. At the 60 min time point glucose (20 %) was infused at a rate of 3 mg/kg over 1 min followed by a 9 min pause every 10 min from time 60 min to 150 min by a programmable Harvard pump (Harvard Apparatus, Mass., USA). Blood was collected every minute from time 90 to 150 min to establish serum insulin concentration time-series.

Sampling. 1.5 ml blood was collected over 15 s every min from time 0 to 60 min and from 90 to 150 min for measurements of serum insulin. The sampling catheter was perfused with saline (0.9 %, 1.0 ml/min) and twenty seconds before sampling, the saline infusion was stopped and 1.0 ml blood was drawn to correct for dead space. After sampling the saline infusion was resumed.

At day 6 a first-phase insulin secretion test was carried out. Injecting glucose (25 g) intravenously from time 0 to 1 min with blood collected at time 0, 2, 4, 6, 8, 10, 12 and 15 min to measure plasma glucose, serum insulin and serum C-peptide. In five of the subjects an OGTT (75 g glucose) was carried out at day 7 after the entrainment period. Not all of the subjects could have an OGTT because of late approval of the protocol amendment. Blood for assessment of plasma glucose, serum insulin and serum C-peptide was collected at time 0, 15, 30, 60, 90 and 120 min.

Assays. Plasma glucose concentrations were measured in duplicate by the glucose oxidation method (Beckman Instruments, Palo Alto, Calif., U.S.A.). Serum insulin concentrations were measured in duplicate by a two-site immuno-specific insulin ELISA, which uses two monoclonal antibodies (DAKO Diagnostics, Cambridgeshire, UK) specific for human insulin. The detection range of this insulin ELISA was 5 pmol/l to 600 pmol/l with an interassay coefficient of variation of 3 %. There was no cross reactivity with proinsulin and split (32, 33)- and des (31, 32)-proinsulin, whereas the antibodies cross reacted (45 % and 66 %) with split (65–66)-proinsulin and des (64, 65)-proinsulin, respectively. No cross-reactivity was obtained with C-peptide, IGF-I, IGF-II and glucagon [21]. Serum C-peptide was measured using a commercially available ELISA kit (K6218, DAKO Diagnostics). Plasma glucagon was measured by a specific RIA which not react with intestinal proglucagon-derived molecules. Serum NEFA concentrations were measured by a colorimetric method using a commercial kit (WAKO Chemicals, Neuss Germany).

Analytical strategy. Spectral and autocorrelation analysis require stationary time-series. An eleven-point centred, unweighted moving average was calculated and subtracted from the original time-series. Analyses were done on the residuals. This detrending method was used to preserve pulsatility with a periodicity near 10 min, which would be expected both spontaneously and after the glucose-entrainment protocol [20].

Spectral analysis was used to quantify the degree of periodicity in a given time series. A Tukey window of 25 data points was used and the spectra were normalized with the assumption that the total variance in each time series was 100 %. The effect parameter at the baseline assessment was the maximal spectral power occurring at any periodicity. During glucose-pulse-entrainment the effect parameter was the spectral power at 10 min. The spectral analysis was done by using noncommercial software.

Autocorrelation analysis was carried out by using the statistical software package SPSS version 9.0 (SPSS, Chicago, Ill., USA). Likewise the maximal autocorrelation coefficient was

Table 1. Clinical characteristics of the subjects during placebo and prednisolone treatment

	Placebo	Prednisolone	
Fasting values			
Serum insulin concentration (pmol/l)	27 ± 3	44 ± 5	**
Plasma glucose concentration (mmol/l)	5.0 ± 0.1	5.5 ± 0.2	*
Serum C-peptide (pmol/l)	461 ± 54	815 ± 48	**
Serum FFA (mmol/l)	0.56 ± 0.16	0.53 ± 0.07	
HOMA-S	1.0 ± 0.1	1.9 ± 0.3	**
First-phase			
Serum insulin AUC (pmol/l per 10 min)	1688 ± 207	3016 ± 468	*
OGTT			
Serum insulin AUC (pmol/l per 120 min)	20033 ± 3123	36918 ± 3024	**
Plasma glucose AUC (mmol/l per 120 min)	797 ± 56	862 ± 49	
Plasma glucose 120 min (mmol/l)	5.2 ± 0.2	6.5 ± 0.4	*
Serum insulin 120 min (pmol/l)	79 ± 11	185 ± 26	*

Data are given as the means ± SEM
*$p < 0.05$
**$p < 0.005$

calculated at baseline while regularity during glucose-pulse-entrainment was determined as the correlation coefficient at a time lag of 10 min.

Deconvolution analysis was used to calculate basal insulin secretion together with mass and amplitude of insulin secretory bursts from plasma insulin concentration profiles [22].

Homeostasis model assessment of insulin sensitivity (HOMA-S) was calculated from fasting serum insulin and fasting plasma glucose [23].

Using peak-insulin concentrations during the first-phase insulin secretion test and correlating them with HOMA-S, we calculated an analogue to the disposition index [24].

Statistical analysis. All data in the text and figures are given as means ± SEM and a p value of less than 0.05 was considered significant unless stated otherwise. A Student's t test was used to evaluate statistical significance. Statistical comparisons were carried out using the statistical software package SPSS version 9.0 (SPSS). Total area under the curve (AUC) for the first-phase insulin secretion and oral glucose tolerance test was calculated using the trapezoidal rule.

Results

Serum insulin, C-peptide, NEFA and plasma glucose concentrations. Fasting serum insulin and serum C-peptide concentrations ($p < 0.005$) and plasma glucose ($p < 0.05$) were higher in the prednisolone period and correlated well with increased HOMA-S (decreased insulin sensitivity) compared to the placebo period ($p < 0.005$). Fasting serum NEFA and plasma glucagon did not differ between the two periods ($p > 0.1$) (Table 1).

First-phase insulin secretion and OGTT. The total area under the curve (AUC) during first-phase insulin secretion in the prednisolone period increased significantly compared to the placebo period (3016 ± 207 vs 1688 ± 468 pmol/l ≅ per 10 min; $p < 0.01$). In addition AUC_{ins} during the OGTT were higher during prednisolone than during the placebo treatment ($p < 0.05$), as were plasma glucose ($p < 0.01$) and serum insulin ($p < 0.05$) concentrations at time 120 min. The OGTT carried out in five subjects remained within the normal range during both treatments.

Disposition index. To assess a disposition index analogue, we expressed peak insulin during the first-phase insulin secretion test to the correlating HOMA-S (Fig. 1) which showed the well known hyperbolic curve [24, 25], indicating that insulin resistance was compensated for by augmented insulin secretion. The calculated disposition index showed no difference between placebo or prednisolone treatment (318.7 ± 24.4 vs 308.2 ± 44.2; $p = 0.79$). There was no correlation between the changes in the disposition index and in pulsatility estimated as insulin secretory burst mass or in the maximal spectral power during basal conditions ($p > 0.5$).

Spectral analysis. During baseline, maximal normalized spectral power of observed spontaneously oscillatory insulin peaks was different (~30%) in the two groups with lower maximal normalized spectral power after prednisolone treatment (prednisolone: 8.40 ± 0.95; range 4.85 ± 13.4 vs placebo 11.79 ± 1.66; range 5.86 – 17.87% total power; $p < 0.05$), indicating a more irregular pulsatile pattern of insulin. After pooling the individual spectra we found a significant decrease in spectral power in the area of 12 to 17 min ($p < 0.05$) after prednisolone treatment (Fig. 2).

No difference was observed in the maximal normalized spectral power of insulin peaks at the time point 10 min during glucose-pulse-entrainment (prednisolone: 13.75 ± 1.77; range 4.53 ± 20.4 vs placebo: 15.75 ± 1.55; range 7.19 – 20.85% total power; $p = 0.34$) and no difference was observed when the in-

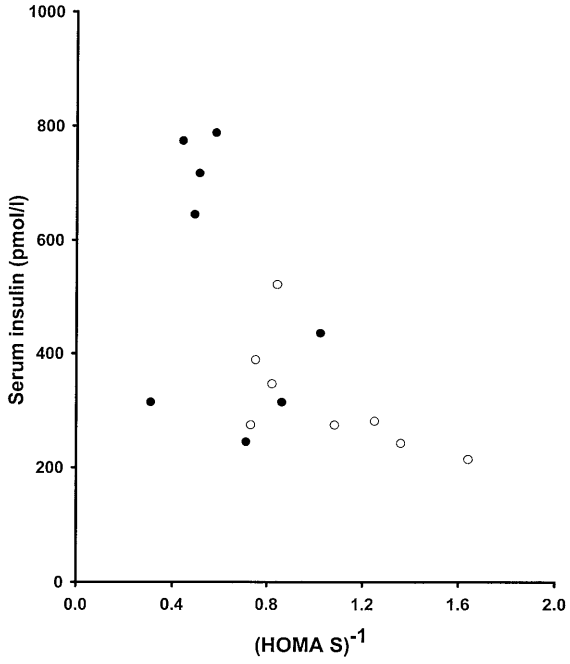

Fig. 1. The relation between peak serum insulin concentrations during the first-phase insulin secretion test and insulin resistance $(HOMA\text{-}S)^{-1}$ is shown to express a disposition index equivalent. (○ placebo, ● prednisolone)

dividual spectra were pooled ($p > 0.05$) between the two treatment periods (Fig. 2).

Applying the autocorrelation analysis to the insulin time-series during basal and glucose-pulse-entrainment showed no statistically significant difference between placebo and prednisolone treatment but there was a trend towards lower autocorrelation coefficients in the basal state during prednisolone treatment than during the placebo conditions (prednisolone: 0.23 ± 0.04; range 0.08 – 0.36 vs placebo: 0.32 ± 0.07; range 0.06 – 0.57, $p = 0.12$).

Pulsatile insulin secretion calculated by deconvolution analysis did not show any alteration in the basal state nor during entrainment measured by insulin secretory burst mass and burst amplitude during the two periods ($p > 0.05$). However secretory burst mass and amplitude tended to be increased during prednisolone treatment ($p = 0.066$ and $p = 0.062$) during the glucose-pulse-entrainment period. Basal insulin secretion was not different during the placebo and the prednisolone treatment ($p = 0.13$) (Table 2).

One representative example of insulin concentration time-series during baseline and glucose-pulse-entrainment after placebo and prednisolone treatment is shown in Figure 3.

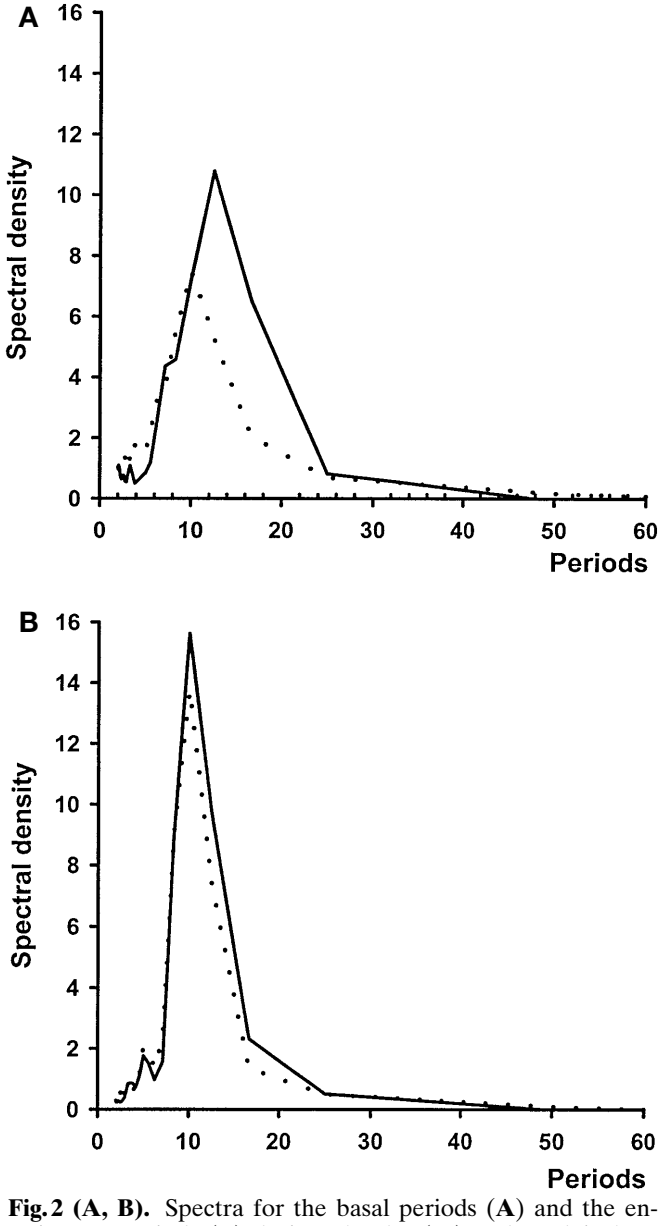

Fig. 2 (A, B). Spectra for the basal periods (**A**) and the entrainment periods (**B**) during placebo (—) and prednisolone treatment (· · ·). The periods were significantly different in the area 12–17 min in the basal state ($p < 0.05$) with lower spectral power after prednisolone treatment whereas no difference could be found in the entrainment period ($p > 0.05$)

Discussion

We sought to evaluate the impact of glucocorticoid-induced insulin resistance on the regularity of the baseline and glucose-pulse-entrained high-frequency insulin pulsatility. We found that short-term glucocorticoid treatment, and hence insulin resistance, results in a perturbed high-frequency insulin pulsatility in the fasting state, whereas the ability of low dose glucose to entrain insulin secretion seems to be preserved.

In vitro studies have reported diverging (stimulating, inhibitory and unchanged) effects of glucocorticoids on the beta cell, depending on the amount and duration of exposure [26–28]. Several studies have, however, shown a clear direct inhibitory effect on glu-

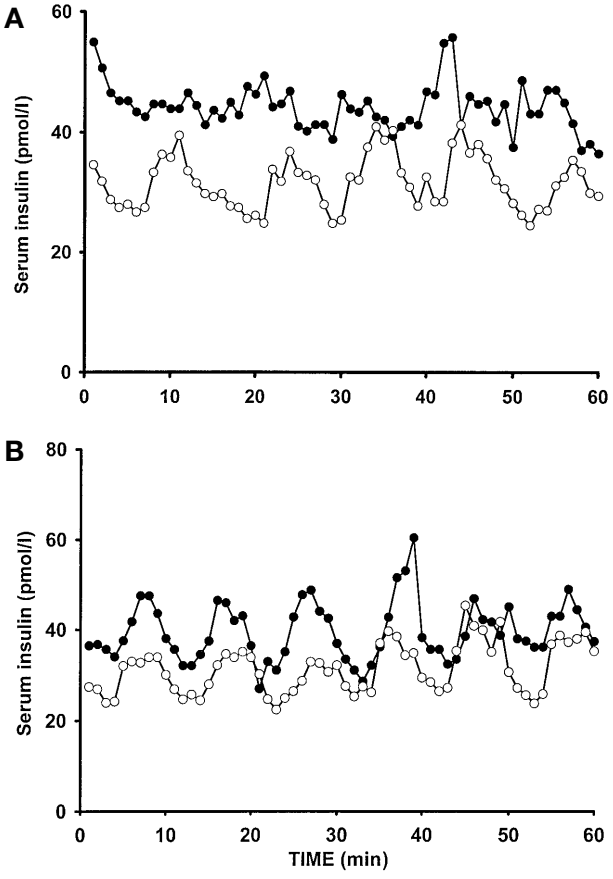

Fig. 3 (A, B). Insulin time series for the basal period (**A**) and the glucose entrainment period (**B**) (○ placebo, ● prednisolone) in a representative subject. Note the deterioration of high-frequency insulin pulsatility during prednisolone treatment in the basal state

cose-stimulated insulin secretion by glucocorticoids during short-term [29–31] and long-term [32] exposure of beta cells. One study [32] suggested that this inhibition of insulin secretion could be mediated through the decreased effectiveness of cytoplasmatic calcium on the secretory process upon stimuli. These findings have not been clearly reproduced in vivo, whereby glucorticoid exposure has been shown to enhance insulin secretion [33–37].

It has been established that insulin secretion is mainly regulated by the pulsatile component of insulin secretion, mostly through pulsatile secretory burst mass and amplitude [22, 38]. We were not able to detect any difference in insulin secretory burst mass, amplitude and basal insulin secretion between placebo and prednisolone treatment, although a marked basal hyperinsulinaemia and increased insulin resistance during prednisolone treatment was shown. A possible explanation for this finding could be the large variation in pulsatile insulin secretion in response to prednisolone treatment between subjects.

Both insulin resistance and impaired beta-cell function are early manifestations in the development of Type II diabetes [9]. In the search for primary defects, studies have been done on first-degree relatives of Type II diabetic patients, along with studies in populations with an extremely high prevalence of diabetes (i.e. Pima Indians) [9, 10]. Both defects are described in these predisposed groups but whether one precipitates the other is still debated. A fundamental characteristic of normal beta-cell function is a highly dynamic pulsatile pattern of insulin release. This pattern exists in individual beta cells, islets, the isolated perfused pancreas, and in vivo. The preservation of a common pulsatile pattern throughout the organisation of the insulin secreting cell population indicates a crucial role of this pattern for beta-cell function and/or for optimal insulin action [39, 40]. Since this pattern is disrupted in glucose tolerant [12] and glucose intolerant [13] first-degree relatives of Type II diabetic patients, a primary defect in the oscillatory secretion has been speculated. However, these subjects are also insulin resistant and the observed beta-cell defects might be secondary. Since minimal glucose excursions occur in the peripheral circulation, and since similar excursions could control the pulsatile insulin secretion [16], it is likely that a feedback loop including pulsatile insulin secretion and oscillatory endogenous glucose production is involved in the regulation of pulsatile insulin secretion in vivo. This study was specifically designed to address whether insulin resistance per se could perturb pulsatile insulin secretion and if so, whether this has a direct effect on the beta cell (i.e. impaired entrainment) or whether the impairment occurs in the basal non-entrained state but with a normal ability to be controlled by the pulsatile glucose infusion. In the lat-

Table 2. Insulin secretion characteristics based on deconvolution analysis during basal and entrainment conditions after placebo and prednisolone treatment

	Placebo		Prednisolone	
	basal	entrainment	basal	entrainment
Deconvolution analysis				
Secretion burst mass (pmol/l/pulse)	19.7 ± 8.8	25.1 ± 11.1	20.3 ± 19.9	36.9 ± 17.3*
Secretion burst amplitude (pmol/l/min)	7.8 ± 3.5	9.8 ± 4.1	8.1 ± 7.9	14.7 ± 6.9*
Basal secretion (pmol/l/min)	3.0 ± 2.0	2.4 ± 1.7	5.3 ± 2.0*	3.5 ± 1.5

Data are means ± SD
* $0.05 < p < 0.15$ testing for treatment effect (placebo vs prednisolone)

ter case, this could suggest a defect in the normal feedback loop, probably involving impaired glucose oscillations and that impaired insulin pulsatility could be secondary.

Since our data show impairment in the basal state, with preserved pulsatility during entrainment, short-term glucocorticoid-induced insulin resistance seems to interfere with the normal feedback dependent system, whereas no direct defect on the beta cell to detect and respond to minimal glucose excursions has been observed. This is further supported by the enhanced peak-insulin in the first-phase insulin secretion test and the stable disposition index. Together, this makes direct effects of prednisolone on the beta cell an unlikely explanation of our observations. The difference between impaired pulsatility at basal conditions, where an intact feedback system is operating, and the normalization when frequency and mass of glucose excursions are controlled exogenously, clearly indicate that the mechanism for prednisolone induced impairment resides in the intact feedback system and is not related to direct beta-cell effects. Since the subjects were not predisposed to diabetes, a difference could occur in healthy subjects who are genetically predisposed to diabetes. Nonetheless, our data suggest that the presence of impaired insulin pulsatility in the basal state could be caused by insulin resistance and thus be secondary. In contrast, no impairment has been reported in obese (insulin-resistant) subjects [41] but one plausible explanation could be a dependency on the duration of insulin resistance and mechanism and/or the site of insulin resistance (i. e. hepatic vs peripheral tissue insulin resistance).

In conclusion, we have shown that glucocorticoid-induced insulin resistance per se could cause impaired insulin pulsatility, probably by a disruption of the normal feed back system. However, direct actions of prednisolone on beta-cell function cannot be ruled out although preserved entrainment favours the former. Glucose-pulse-entrainment as a beta-cell test seems robust to insulin resistance and therefore in settings of variable insulin sensitivity it seems more suitable for searching early beta-cell dysfunction.

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References

1. Polonsky KS, Given BD, Hirsch LJ et al. (1988) Abnormal patterns of insulin secretion in non-insulin-dependent diabetes mellitus. N Engl J Med 318: 1231–1239
2. Lang DA, Matthews DR, Burnett M, Ward GM, Turner RC (1982) Pulsatile, synchronous basal insulin and glucagon secretion in man. Diabetes 31: 22–26
3. Porte DJ (1991) Banting lecture 1990. Beta-cells in type II diabetes mellitus. Diabetes 40: 166–180
4. DeFronzo RA (1988) Lilly lecture 1987. The triumvirate: beta-cell, muscle, liver. A collusion responsible for NIDDM. Diabetes 37: 667–687
5. Pimenta W, Korytkowski M, Mitrakou A, Jenssen T, Yki Jarvinen H, Evron W, Dailey G, Gerich J (1995) Pancreatic beta-cell dysfunction as the primary genetic lesion in NIDDM. Evidence from studies in normal glucose-tolerant individuals with a first-degree NIDDM relative. JAMA 273: 1855–1861
6. Lillioja S, Mott DM, Spraul M et al. (1993) Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus. Prospective studies of Pima Indians. N Engl J Med 329: 1988–1992
7. Ferrannini E (1998) Insulin resistance versus insulin deficiency in non-insulin-dependent diabetes mellitus: problems and prospects. Endocr Rev 19: 477–490
8. Gerich JE (1998) The genetic basis of Type II diabetes mellitus: impaired insulin secretion versus impaired insulin sensitivity. Endocr Rev 19: 491–503
9. Martin BC, Warram JH, Krolewski AS, Bergman RN, Soeldner JS, Kahn CR (1992) Role of glucose and insulin resistance in development of Type II diabetes mellitus: results of a 25-year follow-up study. Lancet 340: 925–929
10. Bogardus C (1993) Insulin resistance in the pathogenesis of NIDDM in Pima Indians. Diabetes Care 16: 228–231
11. Cavaghan MK, Ehrmann DA, Polonsky KS (2000) Interactions between insulin resistance and insulin secretion in the development of glucose intolerance. J Clin Invest 106: 329–333
12. Schmitz O, Porksen N, Nyholm B et al. (1997) Disorderly and nonstationary insulin secretion in relatives of patients with NIDDM. Am J Physiol 272: E218–E226
13. O'Rahilly S, Turner RC, Matthews DR (1988) Impaired pulsatile secretion of insulin in relatives of patients with non-insulin-dependent diabetes. N Engl J Med 318: 1225–1230
14. Peiris AN, Stagner JI, Vogel RL, Nakagawa A, Samols E (1992) Body fat distribution and peripheral insulin sensitivity in healthy men: role of insulin pulsatility. J Clin Endocrinol Metab 75: 290–294
15. Hunter SJ, Atkinson AB, Ennis CN, Sheridan B, Bell PM (1996) Association between insulin secretory pulse frequency and peripheral insulin action in NIDDM and normal subjects. Diabetes 45: 683–686
16. Porksen N, Juhl C, Hollingdal M, Pincus SM, Sturis J, Veldhuis JD, Schmitz O (2000) Concordant induction of rapid in vivo pulsatile insulin secretion by recurrent punctuated glucose infusions. Am J Physiol Endocrinol Metab 278: E162–E170
17. Chou HF, Ipp E (1990) Pulsatile insulin secretion in isolated rat islets. Diabetes 39: 112–117
18. Sturis J, Pugh WL, Tang J, Ostrega DM, Polonsky JS, Polonsky KS (1994) Alterations in pulsatile insulin secretion in the Zucker diabetic fatty rat. Am J Physiol 267: E250–E259
19. Mao CS, Berman N, Roberts K, Ipp E (1999) Glucose entrainment of high-frequency plasma insulin oscillations in control and type 2 diabetic subjects. Diabetes 48: 714–721
20. Hollingdal M, Juhl CB, Pincus SM et al. (2000) Failure of physiological plasma glucose excursions to entrain high-frequency pulsatile insulin secretion in Type II diabetes. Diabetes 49: 1334–1340
21. Andersen L, Dinesen B, Jorgensen PN, Poulsen F, Roder ME (1993) Enzyme immunoassay for intact human insulin in serum or plasma. Clin Chem 39: 578–582
22. Porksen N, Munn S, Steers J, Vore S, Veldhuis J, Butler P (1995) Pulsatile insulin secretion accounts for 70 % of total

insulin secretion during fasting. Am J Physiol 269: E478–E488
23. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28: 412–419
24. Kahn SE, Prigeon RL, McCulloch DK et al. (1993) Quantification of the relationship between insulin sensitivity and beta-cell function in human subjects. Evidence for a hyperbolic function. Diabetes 42: 1663–1672
25. Larsson H, Ahren B (2000) Islet dysfunction in insulin resistance involves impaired insulin secretion and increased glucagon secretion in postmenopausal women with impaired glucose tolerance. Diabetes Care 23: 650–657
26. Kawai A, Kuzuya N (1977) On the role of glucocorticoid in glucose-induced insulin secretion. Horm Metab Res 9: 361–365
27. Ogawa A, Johnson JH, Ohneda M et al. (1992) Roles of insulin resistance and beta-cell dysfunction in dexamethasone-induced diabetes. J Clin Invest 90: 497–504
28. O'Brien TD, Westermark P, Johnson KH (1991) Islet amyloid polypeptide and insulin secretion from isolated perfused pancreas of fed, fasted, glucose-treated, and dexamethasone-treated rats. Diabetes 40: 1701–1706
29. Billaudel B, Sutter BC (1979) Direct effect of corticosterone upon insulin secretion studied by three different techniques. Horm Metab Res 11: 555–560
30. Barseghian G, Levine R (1980) Effect of corticosterone on insulin and glucagon secretion by the isolated perfused rat pancreas. Endocrinology 106: 547–552
31. Barseghian G, Levine R, Epps P (1982) Direct effect of cortisol and cortisone on insulin and glucagon secretion. Endocrinology 111: 1648–1651
32. Lambillotte C, Gilon P, Henquin JC (1997) Direct glucocorticoid inhibition of insulin secretion. An in vitro study of dexamethasone effects in mouse islets. J Clin Invest 99: 414–423
33. Hosker JP, Burnett MA, Matthews DR, Turner RC (1993) Prednisolone enhances beta-cell function independently of ambient glycemic levels in type II diabetes. Metabolism 42: 1116–1120
34. Ludvik B, Clodi M, Kautzky-Willer A et al. (1993) Effect of dexamethasone on insulin sensitivity, islet amyloid polypeptide and insulin secretion in humans. Diabetologia 36: 84–87
35. Louard RJ, Bhushan R, Gelfand RA, Barrett EJ, Sherwin RS (1994) Glucocorticoids antagonize insulin's antiproteolytic action on skeletal muscle in humans. J Clin Endocrinol Metab 79: 278–284
36. 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
37. Tounian P, Schneiter P, Henry S, Delarue J, Tappy L (1997) Effects of dexamethasone on hepatic glucose production and fructose metabolism in healthy humans. Am J Physiol 273: E315–E320
38. Porksen N, Nyholm B, Veldhuis JD, Butler PC, Schmitz O (1997) In humans at least 75 % of insulin secretion arises from punctuated insulin secretory bursts. Am J Physiol 273: E908–E914
39. Lang DA, Matthews DR, Peto J, Turner RC (1979) Cyclic oscillations of basal plasma glucose and insulin concentrations in human beings. N Engl J Med 301: 1023–1027
40. Schmitz O, Pedersen SB, Mengel A et al. (1994) Augmented effect of short-term pulsatile versus continuous insulin delivery on lipid metabolism but similar effect on whole-body glucose metabolism in obese subjects. Metabolism 43: 842–846
41. Sonnenberg GE, Hoffman RG, Mueller RA, Kissebah AH (1994) Splanchnic insulin dynamics and secretion pulsatilities in abdominal obesity. Diabetes 43: 468–477