Disruption of Circadian Insulin Secretion Is Associated With Reduced Glucose Uptake in First-Degree Relatives of Patients With Type 2 Diabetes

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The objective of this study was to evaluate whether first-degree relatives (FDRs) of patients with type 2 diabetes had abnormal circadian insulin secretion and, if so, whether this abnormality affected their glucose metabolism. Six African-American FDRs with normal glucose tolerance and 12 matched normal control subjects (who had no family history of diabetes) were exposed to 48 h of hyperglycemic clamping (~12 mmol/l). Insulin secretion rates (ISRs) were determined by deconvolution of plasma C-peptide levels using individual C-peptide kinetic parameters. Detrending and smoothing of data (*z*-scores) and computation of autocorrelation functions were used to identify ISR cycles. During the initial hours after start of glucose infusions, ISRs were ~60% higher in FDRs than in control subjects (585 vs. 366 nmol/16 h, $P < 0.05$), while rates of glucose uptake were the same (5.6 mmol · kg^{-1} · h^{-1}), indicating that the FDRs were insulin resistant. Control subjects had well-defined circadian (24 h) cycles of ISR and plasma insulin that rose in the early morning, peaked in the afternoon, and declined during the night. In contrast, FDRs had several shorter ISR cycles of smaller amplitude that lacked true periodicity. This suggested that the lack of a normal circadian ISR increase had made it impossible for the FDRs to maintain their compensatory insulin hypersecretion beyond 18 h of hyperglycemia. As a result, ISR decreased to the level found in control subjects, and glucose uptake fell below the level of control subjects (61 vs. 117 µmol · kg^{-1} · min^{-1}, $P < 0.05$). In summary, we found that FDRs with normal glucose tolerance had defects in insulin action and secretion. The newly recognized insulin secretory defect consisted of disruption of the normal circadian ISR cycle, which resulted in reduced insulin secretion (and glucose uptake) during the ascending part of the 24 h ISR cycle. *Diabetes* 48:2182–2188, 1999

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AUC, area under the curve; FDR, first-degree relative; GIR, glucose infusion rate; G_{Rd}, rate of isotopically determined glucose disappearance; ISR, insulin secretion rate.

First-degree relatives (FDRs) of patients with type 2 diabetes develop diabetes at a greater rate than the normal population (1–3) and have been used to uncover early abnormalities that may be important in the development of type 2 diabetes. For instance, several studies have shown that in glucose-tolerant FDRs, defects in insulin action precede defects in insulin secretion (1,4–8). Others, however, have shown the opposite: namely, defects in insulin secretion preceding defects in insulin action (9). Hence, the question remains unsettled as to whether insulin resistance or insulin deficiency is the primary pathogenetic event in type 2 diabetes.

We have recently demonstrated that normal subjects have circadian (24 h) cycles of insulin secretion and plasma insulin levels (rising in the early morning, peaking in the afternoon, and declining during the night) (10), which were different from the earlier recognized short (10–14 min) and longer (1–3 h) pulsatile cycles of insulin secretion (11–13). These circadian cycles of insulin secretion rate (ISR) and plasma insulin levels were absent in patients with type 2 diabetes (14). It is currently not known whether the absence of circadian ISR cycles in type 2 diabetes is a secondary phenomenon, i.e., a consequence of the multiple metabolic and hormonal abnormalities of type 2 diabetes, or whether it is a primary, and perhaps pathophysiologically, important phenomenon.

The goal of this study was, therefore, to determine whether FDRs have abnormal circadian insulin secretion and, if so, whether this could affect their glucose metabolism. To this end, we have measured ISR and glucose uptake rates during 48 h of hyperglycemic clamping in FDRs with normal glucose tolerance and in matched normal control subjects. Hyperglycemia was used because it facilitates recognition of circadian ISR periodicity (10).

RESEARCH DESIGN AND METHODS

Subjects. We have studied six African-American FDRs. Of these, four had one and the other two had two parents with type 2 diabetes. In addition, we have studied 12 African-American control subjects without a family history of diabetes. The control subjects were divided into two groups. Group 1 ($n = 8$) was matched for sex, weight, and age with the FDRs. Group 2 ($n = 4$) was matched with FDRs for sex, weight, and age, as well as for ISR (Table 1). All study subjects had normal oral glucose tolerance tests (Fig. 1); none were taking any medications or participated in regular exercise programs. Their weights were stable for at least 2 months, and their diets contained a minimum of 250 g of carbohydrate for at least 2 days before the studies. The purpose, nature, and potential risks of the study were explained

TABLE 1
Characteristics of study subjects

	FDRs	Control subjects 1	Control subjects 2	*P*
Sex (M/F)	5/1	7/1	4/0	NS
Age (years)	30 ± 4	34 ± 3	33 ± 5	NS
Height (cm)	177.5 ± 3.4	178.9 ± 3.3	183.0 ± 3.7	NS
Weight (kg)	87.0 ± 7.3	83.1 ± 2.5	89.0 ± 3.5	NS
BMI (kg/m^2)	27.6 ± 2.1	26.2 ± 0.4	26.9 ± 1.3	NS

Control subjects in group 2 had initial ISRs comparable to those of FDRs.

to all subjects, and informed consent was obtained. The protocol was reviewed and approved by the Institutional Review Board of Temple University Hospital.

Experimental design. All subjects were admitted to Temple University Hospital's General Clinical Research Center on the morning of the studies and received a standard breakfast at ~9:00 AM. To test the effects of hyperglycemia on insulin secretion and action, hyperglycemic clamps (~12 mmol/l) were started between 1:00 and 2:00 PM and continued for 48 h. A short polyethylene catheter was inserted into an antecubital vein for infusion of test substances. Another catheter was placed into a contralateral forearm vein for blood sampling. This arm was wrapped with a heating blanket (~70°C) to arterialize venous blood.

Hyperglycemic clamps. A 20% glucose solution was infused at variable rates calculated to maintain plasma glucose at ~12 mmol/l. Plasma glucose was monitored, and infusion rates were adjusted every hour, or more frequently when needed. The patients fasted but were allowed to drink water. Plasma electrolytes were monitored every 6 h, body weight every 12 h, and fluid balance every 6 h. Fluid balance was maintained with infusion of normal saline. Potassium and magnesium were added to the glucose infusions as needed to maintain normal plasma concentrations. Blood samples were collected every 30 min for the initial 2 h of glucose infusion and every 2 h thereafter for measurement of glucose, insulin, and C-peptide.

C-peptide kinetic studies. C-peptide kinetics, needed to calculate insulin secretory rates, were determined several days before the studies. An intravenous bolus of 50 nmol of biosynthetic human C-peptide (Eli Lilly, Indianapolis, IN) was administered to each subject after an overnight fast, and plasma C-peptide was measured at frequent intervals for 3 h as described by Polonsky et al. (13) and Van Cauter et al. (15). Individual kinetic parameters of C-peptide clearance were determined by analysis of the C-peptide decay curves.

ISRs. The C-peptide kinetic parameters were used to calculate insulin secretory rates during the hyperglycemic period by deconvolution of peripheral C-peptide concentrations according to Polonsky et al. (16). Plasma volume was assumed to be 4.1% of ideal body weight plus 1% of excess body weight (17).

Determination of diurnal ISR cycles. ISR data were converted to detrended *z*-scores using robust biweight regression (18).

Detrended *z*-scores were calculated as $(ISR - ISR_{predicted})/SD_{regression}$, where $ISR_{predicted}$ equals ISR from the ISR versus time regression and $SD_{regression}$ equals the standard deviation of the regression line. The autocorrelation function (A) was determined separately for each subject as $A(T) = \int C(t) \cdot C(t + T)dt$, where C is normalized data points, t is the time, and T the lag time of the correlation (19), and was used to determine ISR periodicity. Measurements were smoothed using splines (20). Regression analysis with ISR as the dependent variable, the cosine of time (with periodicity set to 24 h) as the independent variable (21), and first-order autocorrelated errors was performed for each subject.

Glucose turnover. Glucose turnover was determined by isotope dilution analysis before (0 h) and after 24 h of hyperglycemia. After a bolus of 30 µmol/kg, [6,6-$^2H_2$]glucose was infused intravenously for 2 h at a rate of 0.3 µmol · kg^{-1} · min^{-1}. Glucose was isolated from blood drawn at –90, 0, and 30 min for determination of isotope enrichment by gas chromatography mass spectrometry (Model 4610-B; Finnigan Mat, San Jose, CA) (22). Rates of glucose appearance and disappearance were calculated from the isotope enrichment with Steele's equation for non–steady state conditions (23). Underestimation of glucose appearance rates during hyperglycemia was avoided by adding [6,6-$^2H_2$]glucose to the unlabeled glucose infused to maintain hyperglycemia (24). In addition, the rate of glucose infused to maintain the glucose clamp (GIR) was used as an estimation of glucose uptake.

Analytical procedures. Plasma glucose was measured with a glucose analyzer with the glucose oxidase method; serum free insulin was determined by radioimmunoassay after polyethylene glycol precipitation using an antiserum with minimal (<0.2%) cross-reactivity with proinsulin and with des-31,32-proinsulin (Linco Research, St. Charles, MO); C-peptide was determined by radioimmunoassay with an antiserum that cross-reacted <4% with proinsulin (Linco); and proinsulin was determined with an antiserum that cross-reacted 95% with des-31,32-human proinsulin and <0.1% with des-64,65-human proinsulin, C-peptide, and insulin (Linco). Electrolytes were measured at Temple University Hospital's Chemistry Laboratory.

Statistical analysis. All data were expressed as means ± SE. Statistical significance was assessed using analysis of variance and Student's two-tailed t test when indicated. The variations in ISR and insulin concentration with time were analyzed by repeated measures analysis of variance with the SAS program (SAS Institute, Cary, NC). The Wilcoxon test was used to compare the median autocorrelations of FDR and control subjects and to test for the significance of the median of the coefficients of the cosine function of time.

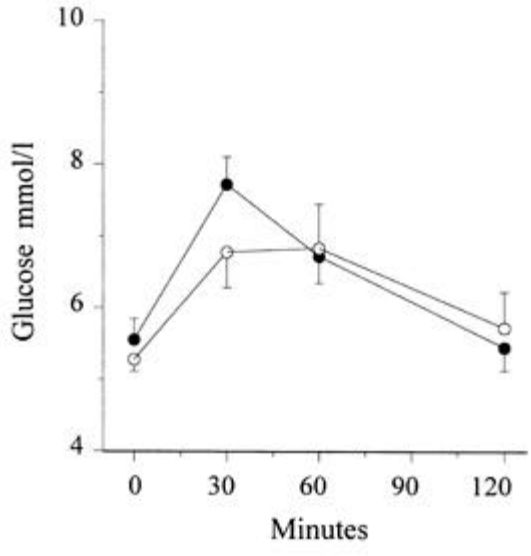

FIG. 1. Plasma glucose concentrations during 2-h oral glucose tolerance tests in 6 FDR (●) and in 12 sex, age, and weight matched normal control subjects (○). All subjects received 75 g of glucose at 0 min. Data shown are means ± SE. The difference at 30 min was not statistically significant.

RESULTS

Basal glucose and insulin levels. Basal plasma glucose concentrations were 4.8 ± 0.2 mmol/l in control subjects and 4.8 ± 0.2 mmol/l in FDRs (NS). Basal serum insulin concentrations were 36 ± 6 pmol/l in control subjects and 60 ± 6 pmol/l in FDRs ($P < 0.05$). Basal C-peptide concentrations were 0.40 ± 0.06 nmol/l in control subjects and 0.62 ± 0.10 nmol/l in FDRs ($P < 0.05$). The basal hyperinsulinemia in FDRs suggested basal insulin resistance.

Insulin secretion during hyperglycemia. Glucose concentrations during the 48-h glucose infusion were the same in FDR and control subjects (11.8 ± 0.2 and 12.0 ± 0.3 mmol/l, respectively, NS) (Fig. 2*A*). During the initial 2 h of hyperglycemia (from 2:00 to 4:00 PM), ISR rose from 67 ± 9 to 442 ± 54 pmol/min ($P < 0.01$) in control subjects and from 94 ± 19 to 736 ± 143 pmol/min ($P < 0.01$) in FDRs. During the initial 16–18 h of hyperglycemia, areas under the ISR curves (AUC ISR) were ~60% larger in FDR than in control subjects (585 ± 98 vs. 366 ± 60 nmol/16 h, $P < 0.05$).

Between 18 and 24 h, ISR remained essentially unchanged in FDRs but rose in control subjects as part of their normal 24-h cycle so that at 24 h, ISRs were the same in control subjects and FDRs (Fig. 2*B*). Between 24 and 48 h, ISRs slowly declined in FDRs, while in control subjects, ISRs first declined and then started to rise again.

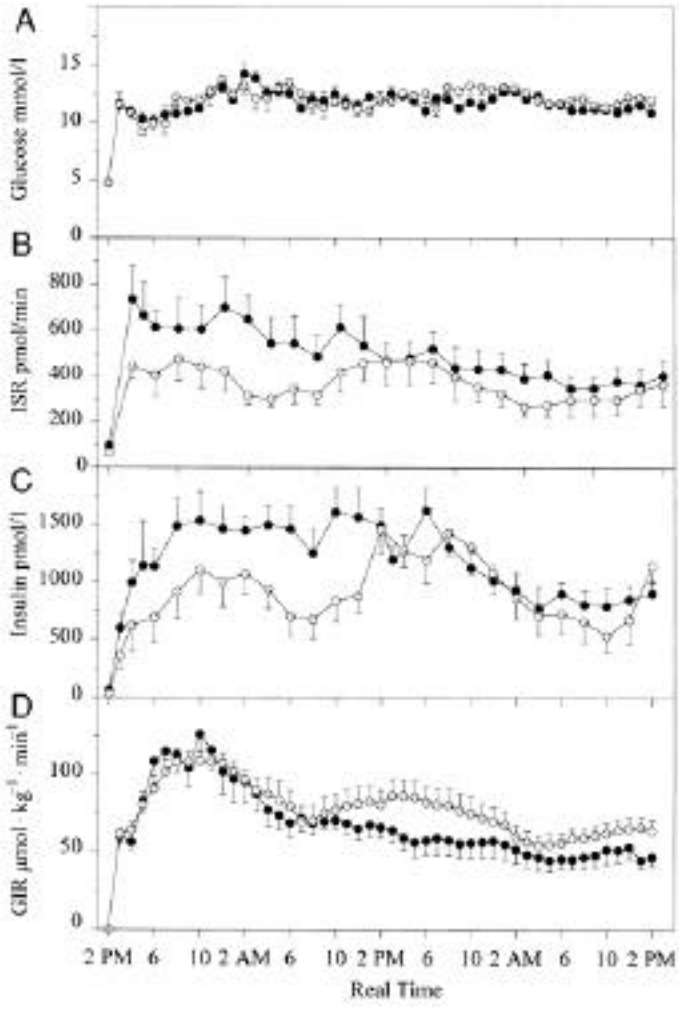

FIG. 2. Plasma glucose and insulin levels and ISRs and GIRs during 48 h of hyperglycemic clamping in six FDRs (●) and eight matched normal control subjects (○). *A*: Glucose; *B*: ISR; *C*: insulin; *D*: GIR.

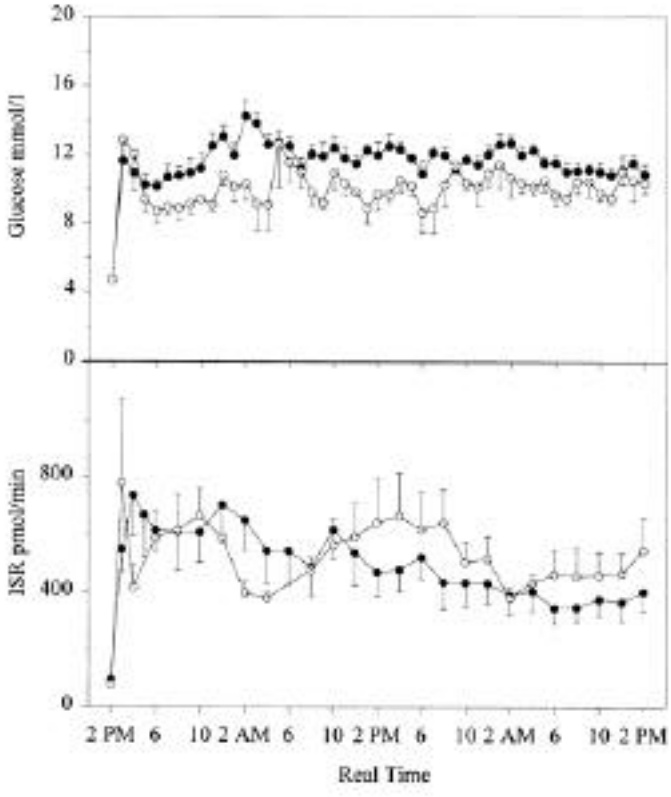

FIG. 3. Plasma glucose levels and ISRs during 48 h of hyperglycemic clamping in four normal control subjects (○) and six FDRs (●). The normal control subjects were selected to have initial ISR comparable to those of the relatives.

Peripheral insulin concentrations followed closely the course of ISRs, rising in response to intravenous glucose and attaining higher concentrations in FDR than in control subjects during the initial 16–18 h (AUC insulin 22.7 ± 2.4 vs. 14.4 ± 2.5 nmol · l^{-1} · 16 h^{-1}, $P < 0.05$), then reaching equal concentrations between 18 and 24 h and declining together between 24 and 48 h (Fig. 2*C*).

The inability of FDRs to raise their ISR after 18 h of hyperglycemia could have been a consequence of unsustainably high ISR. We, therefore, tested four control subjects (Table 1, group 2) whose mean initial ISRs (from 2 to 18 h) were as high as those of the FDRs (511 ± 35 vs. 479 ± 26 pmol/min). In contrast to the FDRs, ISR in these control subjects had a normal circadian increase, starting at ~18 h, demonstrating that ISR in the 600–800 pmol/min range did not disrupt their circadian insulin secretion (Fig. 3).

Glucose uptake. GIR, reflecting insulin plus glucose-stimulated glucose uptake, were the same during the initial 18 h of hyperglycemia in control subjects and FDRs (AUC GIR 5.6 ± 0.3 vs. 5.6 ± 0.6 mmol · kg^{-1} · h^{-1}). When the ISR became equal in FDR and control subjects between 18 and 24 h, GIR in FDRs started to decline relative to control subjects and remained lower in FDR than control subjects between 24 and 48 h (AUC GIR 4.13 ± 0.45 vs. 3.16 ± 0.5 mmol · kg^{-1} · h^{-1}, $P < 0.05$) (Fig. 2*D*).

Rates of isotopically determined glucose disappearance (G_{Rd}) (the calculation of which, unlike GIR, is not affected by residual endogenous glucose production) were similar (72 ± 9 vs. 80 ± 4 µmol · kg^{-1} · min^{-1}, NS) in control subjects and FDRs, respectively, after ~1 h of hyperglycemia. After 24 h of hyperglycemia, however, G_{Rd} was higher in control subjects than in FDRs (117 ± 6 vs. 61 ± 6 µmol · kg^{-1} · min^{-1}, $P < 0.05$), confirming the presence of uncompensated insulin resistance in FDRs.

Circadian rhythmicity of ISR. The temporal pattern of ISR was markedly different in FDR and control subjects. In control subjects, ISR started to rise in the early morning hours, peaked in the early afternoon, and then declined to reach a nadir between 2:00 and 6:00 AM the next day (Figs. 2 and 4). The ISR pattern in FDRs was irregular, the detrended data showing several peaks of smaller amplitude and shorter duration that more or less disappeared upon smoothing (Fig. 4, *upper* and *middle panels*). This abnormal pattern of ISR was seen in all six FDRs (Fig. 5).

The first-order autocorrelation function in control subjects was positive for lags of 1–4 h, then became negative for lags of 4–16 h, and after that reverted to become positive again, suggesting a periodicity of ~24 h. The cosine function of time in the regression model was highly significant ($P < 0.001$), confirming periodicity of ISR (Fig. 4, *lower panel*). In contrast, there was no clear periodicity in FDRs. Mean first-

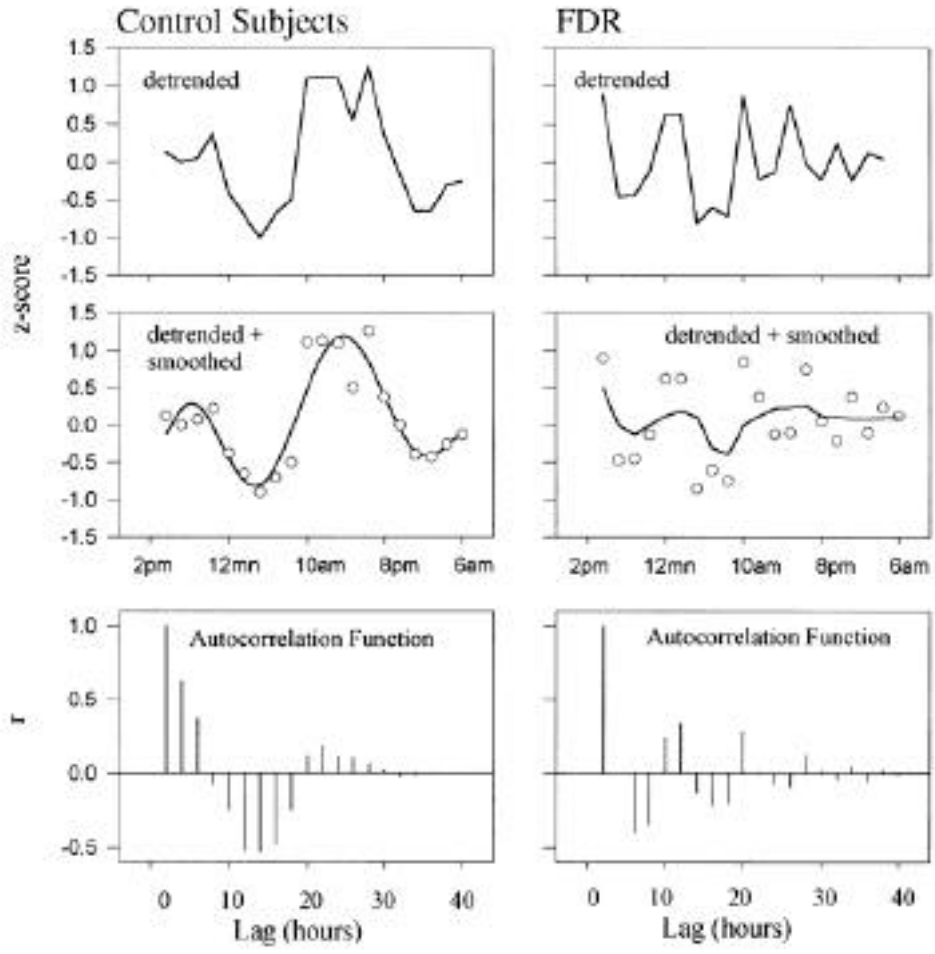

FIG. 4. ISRs in eight normal control subjects (*left panels*) and in six FDRs (*right panels*). Mean *z*-scores of detrended values of the first complete circadian ISR cycle are shown in the upper panels; mean *z*-scores of detrended and smoothed values in the middle panels; and first-order autocorrelation functions in the lower panels.

order autocorrelation in FDRs was close to 0, indicating that there were short-term fluctuations in ISR over 2-h periods (P = 0.01 for the difference in short-term ISR fluctuations between relatives and control subjects). The 24-h autocorrelation was also close to 0, and the autocorrelation function did not have a wave-like appearance, which indicated that there was no true 24-h periodicity. Similar results were obtained with plasma insulin levels (data not shown).

Plasma cortisol and melatonin. Cortisol and melatonin concentrations were determined during one full 24-h cycle to test whether these hormones had maintained their normal circadian rhythmicity. Cortisol and melatonin levels both rose, peaked, and declined similarly in normal control subjects and FDRs (Fig. 6).

Proinsulin. To assess whether hyperglycemic stress could have caused a dysfunction in the processing of proinsulin to insulin, plasma proinsulin and proinsulin-to-insulin ratios were measured serially. Basal proinsulin concentrations were 6.0 ± 2.1 pmol/l in control subjects and 12.0 ± 1.5 pmol/l in FDRs (P < 0.05). Basal molar proinsulin-to-insulin ratios were 0.15 ± 0.04 in control subjects and 0.21 ± 0.03 in FDRs (NS). During hyperglycemia, proinsulin rose in both groups, stabilizing at ~50–60 pmol/l in control subjects and at ~110–120 pmol/l in FDRs. The proinsulin-to-insulin ratios decreased initially during glucose infusion (P < 0.05) (from 0.15 ± 0.04 to 0.04 ± 02 in control subjects and from 0.21 ± 0.03 to 0.06 ± 0.007 in FDRs, P < 0.05) and subsequently rose in both groups, approaching basal rates at the end of the studies (0.13 ± 0.08 and 0.16 ± 0.03 at 44 h). At no time were proinsulin-to-insulin ratios in control subjects and FDRs significantly different from each other (Fig. 7).

DISCUSSION

Disrupted circadian insulin secretion in FDRs. The normal control subjects in this study exhibited well-defined 24-h (circadian) cycles of ISR and insulin levels, rising in the early morning hours, peaking in the early afternoon, and declining during the night, confirming previous findings (10). In contrast, all six FDRs had no circadian cycles of ISR (or of insulin), but instead had several irregular and shorter ISR cycles that lacked true periodicity (Figs. 4 and 5). Similar results were obtained when plasma insulin levels were analyzed (data not shown). The cause for the abnormal patterns of ISR and insulin in FDRs is not known. Numerous publications, however, have demonstrated a strong relationship between the light/dark cycle, plasma melatonin levels, a robust marker of circadian light/dark periodicity (25,26), and circadian biorhythms, including those associated with blood pressure, core body temperature, and plasma cortisol levels (27,28). Moreover, there is strong evidence that nerves, which mono-

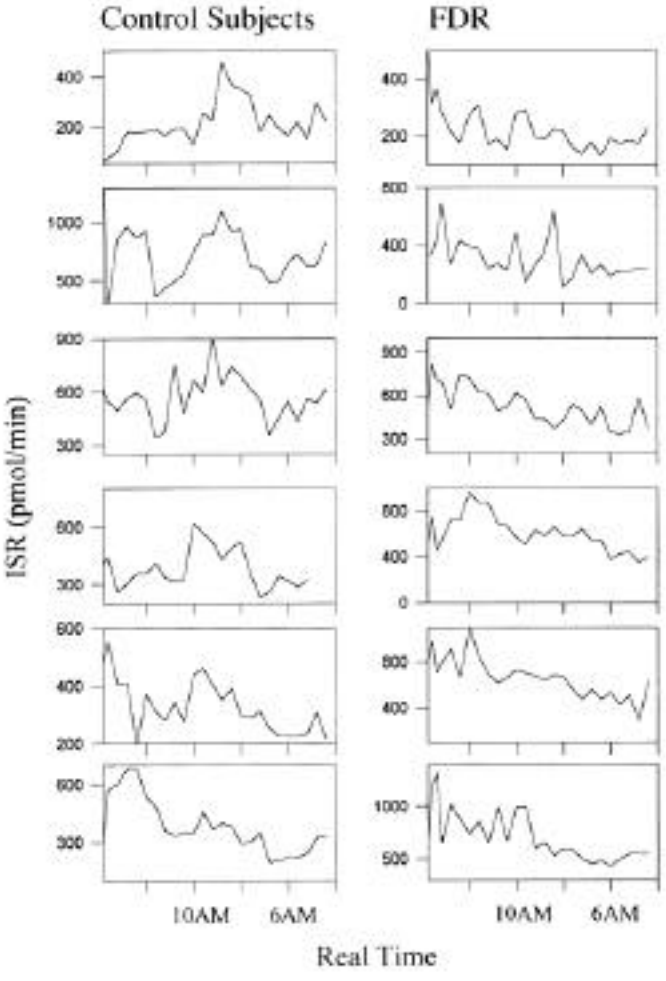

FIG. 5. Individual ISRs before detrending and smoothing in six FDRs (*right panels*) and six control subjects (*left panels*) during 48 h of hyperglycemic clamping.

synaptically connect the retina with suprachiasmatic nuclei in the hypothalamus, are the principal pacemakers of circadian periodicity and that their linkage with the retina mediates the synchronization with the light/dark cycle (28). We have, therefore, measured plasma cortisol and melatonin levels in an attempt to determine whether the abnormal ISR rhythmicity was caused by a central nervous system or a β-cell defect. The finding of normal circadian patterns of plasma cortisol and melatonin levels in FDRs (Fig. 6) suggested that the defect may have been located in their β-cells.

It is interesting that irregularities of pulsatile insulin secretion have been observed previously in FDRs (29). However, since the FDRs in that study were already glucose intolerant, it was not clear whether these pulsatile secretory abnormalities were of primary or secondary nature. Seen in light of the present findings, however, they suggest that there may be a fundamental disturbance of all types of cyclic insulin secretion in FDRs.

The disrupted circadian ISR pattern reduced insulin secretion and glucose uptake. Initially, β-cells of FDRs seemed to function appropriately in response to the acute rise in plasma glucose, secreting a greater than normal amount of insulin to compensate for the underlying insulin resistance. This changed after ~16–18 h, when ISRs rose in control subjects as part of their normal 24 h cycles, while ISRs in FDRs either did not change or declined. By ~24 h, ISR (and plasma insulin) were the same in FDR and control subjects and glucose uptake in FDRs had begun to fall below that of control subjects (Fig. 2). Thus, the absence of a cyclic ISR rise in FDRs at that time appeared to have been responsible for the loss of compensatory insulin oversecretion and the decrease in glucose uptake.

It could be argued that the inability of FDRs to raise their ISR after 18 h was due to the fact that their ISRs were already very high and could not be raised further. In other words, the same would have occurred in normal control subjects, had their initial ISRs been equally as high as those of the FDRs.

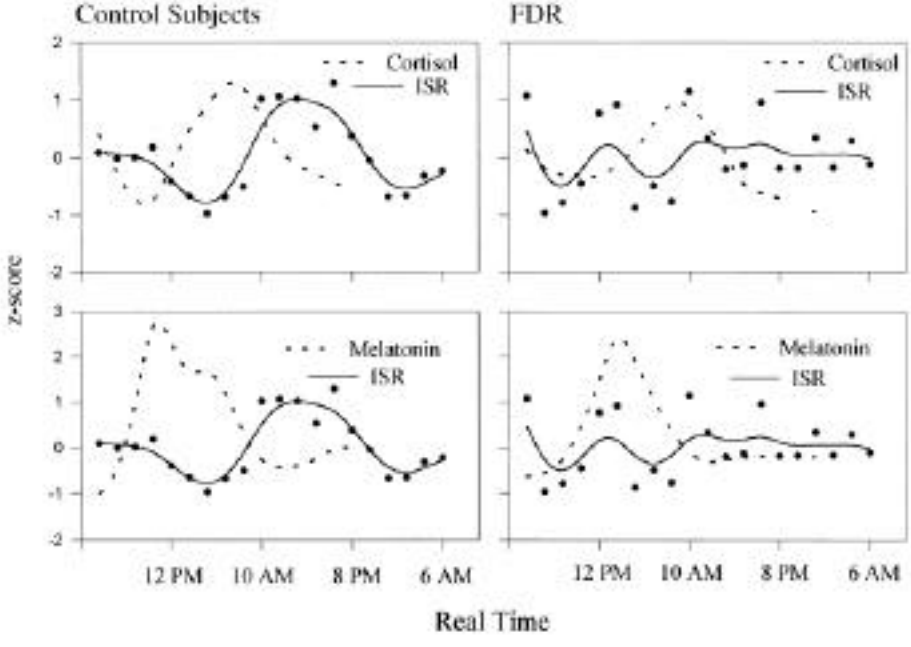

FIG. 6. *z*-scores of smoothed values of plasma cortisol concentrations and ISR (*upper panels*) and of plasma melatonin concentrations and ISR (*lower panels*) during hyperglycemic clamping. Data shown are mean values from one complete circadian cycle.

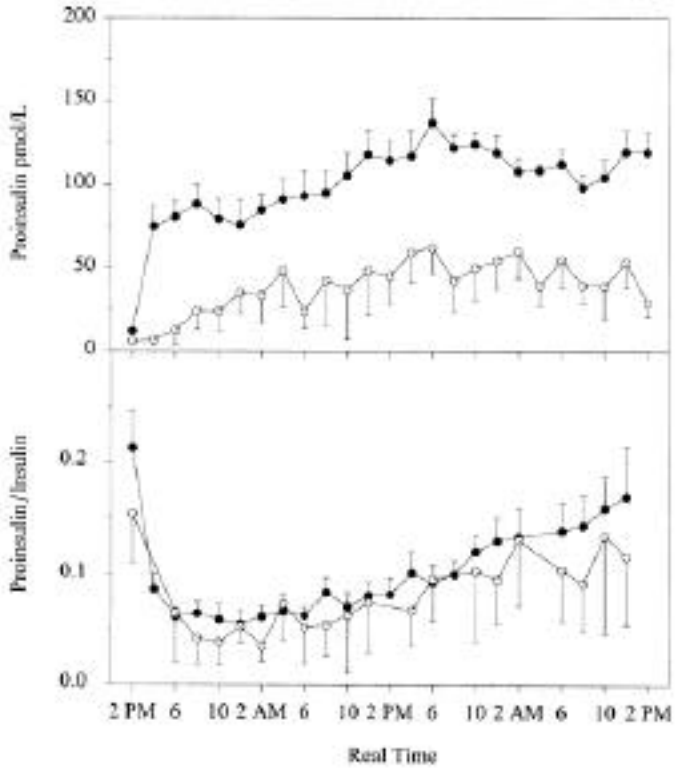

FIG. 7. Plasma proinsulin (plus des-31,32-proinsulin) concentrations and plasma proinsulin-to-insulin ratios during 48 h of hyperglycemic clamping in six FDRs (●) and eight normal control subjects (○).

This possibility seems rather unlikely, since we have demonstrated that a group of normal control subjects who had ISRs as high as the initial ISRs of FDRs were able to maintain their normal circadian ISR cycles for >24 h (Fig. 3). Nevertheless, the possibility cannot be excluded that the FDRs had a reduced β-cell mass (30) and that because of that, secreting the same amount of insulin as control subjects presented increased secretory stress.

The reason that FDRs were able to maintain higher ISR than control subjects during the initial 16–18 h is not clear. It could have been due to the fact that in control subjects, ISR declined during most of this period as part of their normal 24-h ISR cycle. Alternatively, it is possible that 18 h of hyperglycemia had somehow exhausted the β-cells in FDRs (31).

To explore possible reasons for the β-cell dysfunction in FDRs, we considered the possibility that the prolonged hyperglycemia had placed an increased demand on proinsulin processing, which may have become dysfunctional (32). This seemed reasonable because of reports that patients with type 2 diabetes have disproportionally elevated plasma levels of proinsulin and its conversion intermediates, suggesting that the processing of proinsulin to insulin was perturbed (33–35). If this was the case in FDRs, one would expect to see accumulation of proinsulin and its most abundant intermediate metabolite, des-31,32-proinsulin (33,36). This, however, was not the case. Plasma proinsulin (which included des-31,32-proinsulin) was only ~6% of plasma insulin during the first 6 h of hyperglycemia and the proinsulin-to-insulin ratio was similar in FDR and control subjects at all times (Fig. 7). The slow increase in the proinsulin-to-insulin ratios, which occurred throughout hyperglycemia in both groups, was probably caused by the longer half-life of proinsulin compared with insulin (37), which resulted in accumulation of proinsulin (and des-31,32-proinsulin) in the blood. Hence, our studies did not support the notion that prolonged hyperglycemia in FDRs had interfered with processing of proinsulin to insulin.

Lastly, it is necessary to consider what role glucose toxicity/desensitization (38) may have played in the abnormal β-cell function in FDRs. We have previously shown in normal control subjects that the same degree of hyperglycemia as that used here (i.e., ~12 mmol/l) inhibited glucose uptake as well as insulin secretion, i.e., produced glucose toxicity after ~35–40 h (39). Therefore, interpretation of ISR and GIR data after ~35 h of hyperglycemia in this study is complicated by the development of glucose toxicity/desensitization. On the other hand, it is conceivable that β-cells of FDRs were more susceptible than those of control subjects to glucose toxicity/desensitization and that, therefore, glucose desensitization may have been responsible for some of the β-cell dysfunction in FDRs.

Clinical relevance. Here, we have demonstrated an insulin secretory defect in insulin-resistant FDRs. The defect consisted of a disruption of the normal circadian insulin secretion cycle that resulted in subnormal insulin secretion (and glucose uptake) because of absence of the ascending part of the 24-h ISR cycle. The insulin secretory defect was demonstrated under conditions of prolonged hyperglycemia. Whether it also affects insulin secretion under everyday living conditions and whether our findings apply to a larger population of FDRs remains to be shown. The fact that the FDRs all had normal oral glucose tolerance tests does not rule out that the defect in circadian insulin secretion may play an important role in the development of glucose intolerance in FDRs. First, the glucose tolerance tests were done near the nadir (8:00–9:00 AM) of the ISR cycle, i.e., at a time when one would not expect to find differences between FDR and control subjects. In addition, there is some evidence that the secretory defect is progressing from a disrupted (this study) to a completely absent (14) circadian ISR cycle.

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