Evidence for a Circadian Rhythm of Insulin Sensitivity in Patients With NIDDM Caused by Cyclic Changes in Hepatic Glucose Production

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Diurnal variation in insulin sensitivity in patients with NIDDM has long been suspected but has been difficult to document mainly because of the interdependence of changes in glucose and insulin. Stable serum insulin levels during hyperglycemic clamping in patients with NIDDM in the present study provided the opportunity to examine changes in insulin sensitivity unaffected by changes in blood glucose and insulin concentrations. Six patients with NIDDM (four men and two women, BMI 33.9 ± 2.5) underwent hyperglycemic (11.1 mmol/l, ~200 mg/dl) clamping for 72 h. Measured were serum insulin, free fatty acid (FFA), cortisol, and growth hormone concentrations and rates of insulin secretion, insulin clearance, and glucose infusion rate (GIR) needed to maintain hyperglycemia. In addition, five patients (three men and two women, BMI 32.6 ± 0.6) underwent hyperglycemic clamping for 24 h with hourly determinations of hepatic glucose production (HGP) and glucose disappearance rates (G_{Rd}). GIR, reflecting insulin sensitivity, changed rhythmically with a cycle duration of 22.9 ± 1.4 h and an amplitude of 47.8 ± 11.2%. GIR was lowest at 8:31 a.m. (±52 min) and highest at 7:04 p.m. (±58 min). Circadian changes in GIR were completely accounted for by changes in HGP, while G_{Rd} remained unchanged. Plasma levels of FFAs and cortisol also exhibited circadian fluctuations, and their blood levels correlated negatively with GIR (r = −0.72 and −0.64, respectively). We concluded that insulin sensitivity in patients with NIDDM changed with circadian (~24 h) rhythmicity (decreasing during the night and increasing during the day). These changes were unrelated to blood levels of glucose and insulin, insulin clearance, exercise, food intake, and sleep. They were caused by circadian changes in HGP, which in turn were closely correlated with circadian changes in blood FFA and cortisol levels. We believe that recognition of these circadian changes has implications for the diagnosis and the treatment of patients with NIDDM. ***Diabetes*** **45:1044–1050, 1996**

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CV, coefficient of variation; FFA, free fatty acid; GIR, glucose infusion rate; G_{Ra}, glucose appearance rate; G_{Rd}, glucose disappearance rate; GTO, glucose turnover; HGP, hepatic glucose production; ISR, insulin secretory rate; REM, rapid eye movement; RIA, radioimmunoassay.

The existence of diurnal variations in glucose tolerance and insulin sensitivity in normal and diabetic subjects has been suspected for decades. The older literature contains numerous reports of diurnal changes in plasma glucose and in glucose tolerance (1). These reports, however, were often contradictory. Glucose tolerance was found to be better in the morning in some (2,3) and better in the afternoon in others (4,5). More recently, several groups have reported early morning increases in insulin requirements in patients with IDDM, which suggested an early morning decrease in insulin sensitivity or alternatively an increase in insulin clearance (6–8). The short duration of these studies (one night), however, did not permit recognition of diurnal changes. A major problem complicating studies of cyclic changes in insulin sensitivity has been the strong interdependence between glucose concentrations and insulin secretion (1). For instance, if there was a primary change in glucose concentration, it would produce an isodirectional change in insulin, which then would modify the glucose concentration. On the other hand, a primary change in insulin concentration would inversely affect glucose, which would then modify the insulin concentration. Because of this chicken-and-egg situation, it has remained uncertain whether or not there is a truly diurnal rhythm of insulin sensitivity in patients with IDDM or NIDDM.

The findings in the present study that patients with NIDDM, in contrast to normal volunteers (9), have stable insulin levels during hyperglycemic clamping has allowed us to determine whether these patients have cyclic changes of insulin sensitivity unrelated to changes in blood glucose or insulin levels.

RESEARCH DESIGN AND METHODS

Subjects. The clinical characteristics of the patients with NIDDM participating in these studies are shown in Table 1. All patients had been treated with sulfonylurea drugs, and five patients received in addition small doses of NPH insulin (5–15 U) at bedtime. Insulin and all other medications were discontinued at least 1 day before the studies. The patients' weights were stable for at least 2 months, and their diets contained a minimum of 250 g/day carbohydrate for at least 2 days before the studies. Informed written consent was obtained from all subjects after explanation of the nature, purpose, and potential risks of these studies. The study protocol was approved by the Institutional Review Board of Temple University Hospital.

Experimental design. All patients were admitted to Temple University Hospital's General Clinical Research Center 1 day before the studies. The next morning after an overnight fast, 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

TABLE 1
Clinical characteristics of subjects

Glucose clamp (h)	72	24
Sex (M/F)	4/2	3/2
Age (years)	55.5 ± 6.0	62.0 ± 2.4
Weight (kg)	99.6 ± 12.8	91.2 ± 6.4
Height (cm)	169.5 ± 5.9	167.1 ± 6.2
BMI (kg/m^2)	33.9 ± 2.5	32.6 ± 0.6
Duration of NIDDM (years)	9.2 ± 3.6	9.8 ± 4.2

Data are means ± SE.

sampling. This arm was wrapped with a heating blanket (~70°C) to arterialize venous blood. C-peptide was then infused to determine individual C-peptide kinetics. Between 5:00 and 6:00 P.M., the patients ate a standard dinner consisting of 55% carbohydrate, 15% protein, and 30% fat. At midnight, glucose was clamped at the patients' prevailing blood glucose levels (isoglycemic clamp, ~11.1 mmol/l) and continued for 72 h in five patients. In one patient, glucose was clamped for only 36 h. Blood samples were collected every 2 h for determination of hormones and substrates. One to two months later, three of the original six patients and two patients who had not been studied before were admitted to the General Clinical Research Center for a 24-h glucose turnover (GTO) study. After an overnight fast, their plasma glucose was clamped at ~11.1 mmol/l, and blood was collected hourly for 24 h for determination of GTO rates.

Procedures

Hyperglycemic clamps. Six patients (four men and two women) were studied. A 5% glucose solution was infused at variable rates for 72 h to produce plasma glucose concentrations of ~11.1 mmol/l. Blood samples were collected every 10–15 min for the initial 4 h of glucose infusion, every 1 h thereafter for measurement of glucose, and every 2 h for determination of insulin, C-peptide, cortisol, growth hormone, free fatty acids (FFAs), and lactate. The patients fasted during the study, but were allowed to drink water. Bed rest was continued. Lights were turned off at 9:30 P.M. and turned on at 7:00 A.M. the next morning. Sleep was polygraphically monitored during one night. Plasma electrolytes, body weight, and fluid balance were monitored every 6 h. Fluid balance was maintained with infusion of normal saline. Potassium and magnesium were added to the glucose infusion as needed to maintain normal plasma concentrations.

GTO. Five patients (three men and two women) were studied. GTO was determined with [3-^{3}H]glucose. The tracer infusion (40 μCi over 1 min followed by 0.4 μCi/min) was started 90 min before the initiation of the measurements to ensure isotopical equilibration. Glucose was isolated from blood for determination of [3-^{3}H]glucose specific activity as described (10). [3-^{3}H]glucose specific activity remained stable throughout the 24-h studies. Rates of total body glucose appearance (G_{Ra}) and disappearance (G_{Rd}) were calculated using Steele's equation for steady-state conditions (11). G_{Rd} values were corrected for urinary glucose excretion (0.06 ± 0.007 μmol · kg^{-1} · min^{-1}). (G_{Ra} − urinary glucose excretion = G_{Rd}.)

Hepatic glucose production (HGP) was calculated as the difference between the isotopically determined G_{Ra} and the rates of glucose infusion needed to maintain hyperglycemia during the clamps.

C-peptide kinetic studies. An intravenous bolus of 50 nmol of biosynthetic human C-peptide (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 Van Cauter et al. (12). Individual C-peptide kinetic parameters were determined by analysis of the C-peptide decay curves. The C-peptide kinetic parameters were used to calculate the insulin secretory rates (ISRs) for each time interval between successive blood samples by deconvolution of peripheral C-peptide concentrations (12).

Insulin clearance rates were determined for 6-h periods by dividing the areas under the ISR curves by the area under the serum insulin curves.

Sleep recording. Sleep was monitored with a portable computerized polysomnographic system, (Alice 3, Healthdyne, Marietta, GA). The polygraphic records were scored visually at 30-s intervals in sleep stages 1, 2, 3, 4, and rapid eye movement (REM) based on the criteria of Rechtshaffen and Kales (13).

Determination of diurnal rhythms. To identify a rhythmic pattern, we applied a refined first-order Fourier transform to the respective curves of glucose infusion rate (GIR), cortisol, and FFAs. In a first step, data were normalized to their respective mean values. The autocorrelation function $A(T) = \int (C)(t) \cdot C(t + T)\, dt$, where C is the normalized data points, t is the time, and T is the lag time of the correlation, then was applied to each set of normalized data. This function eliminates the background noise and the nonperiodical events in the defined time interval. The frequency (in cycles per 24 h) and the amplitude in the time domain (in percentage variation) of the cyclical phenomena, were then determined using the Fourier transform $F(w) - \Delta t \int A(T) \cdot \cos \cdot (wT)\, dT$, where w is the pulsation frequency and A is the amplitude of the signal (14).

Analytical procedures. Plasma glucose was measured with a glucose analyzer (Beckman, Palo Alto, CA). Serum-free insulin was determined by radioimmunoassay (RIA) after polyethylene glycol precipitation using an antiserum with minimal (<0.2%) cross-reactivity with proinsulin (Linco, St. Charles, MO). Human growth hormone (15) and glucagon (16) were determined by RIA. Cortisol was measured with a kit (Diagnostic, Los Angeles, CA). Plasma FFA concentration was determined with a kit from Wako (Richmond, VA). Lactate and alanine were measured enzymatically (17,18).

Statistical analysis. All data are expressed as means ± SE. Statistical significance was assessed using analysis of variance and Student's two-tailed t test when indicated.

RESULTS

Glucose, insulin, insulin secretion, clearance, and GIR. Mean glucose concentration was 11.9 ± 0.4 mmol/l (215 ± 7 mg/dl) (coefficient of variation [CV] 5.8 ± 0.6%). Mean insulin concentration was 174 ± 18 pmol/l (29 ± 3 μU/ml). Individual serum insulin concentrations were stable throughout the studies in all six patients (Figs. 1 and 2). Mean ISR was 121 ± 23 pmol/min (0.01815 ± 0.00345 U/min or 0.18 mU · kg^{-1} · min^{-1}), and mean insulin clearance was 0.75 ± 0.16 l/min (7.53 ml · kg^{-1} · min^{-1}).

GIR reflected total body insulin sensitivity. Visual inspection of Fig. 1 showed a pulsatile rhythm of GIR. Fourier transform of the GIR data showed a cycle duration of 22.9 ± 1.4 h and a mean amplitude (variation around the mean) of 47.8 ± 11.2% (± 3.8 μmol · kg^{-1} · min^{-1}). GIR was lowest in the morning (8:31 A.M. [±52 min]) and highest in the evening (7:04 P.M. [±58 min]). The differences between trough and peak values were statistically significant for all 3 days ($P < 0.04$, $P < 0.01$, and $P < 0.01$, respectively).

Glucose production and utilization. Under steady-state conditions, GIR is determined by peripheral (muscle) glucose uptake (G_{Rd}) and HGP (Fig. 3). The circadian changes in GIR could have been produced, therefore, either by changes in G_{Rd}, HGP, or a combination of both. To differentiate between these possibilities, we measured GTO at 1-h intervals between ~9:00 A.M. and ~7:00 A.M. the next morning in five patients (three of whom had previously participated in the 72-h clamp studies).

Glucose was clamped at 11.6 ± 0.4 mmol/l (209 ± 7 mg/dl) (CV 7.4%). Serum insulin was 168 ± 12 pmol/l (28 ± 2 μU/ml) and did not change significantly during the 24-h study period. GIR exhibited the same circadian changes that were seen previously during the 72-h clamp studies, rising from 0 to 6.1 ± 1.1 μmol · kg^{-1} · min^{-1} (1.1 ± 0.2 mg · kg^{-1} · min^{-1}) at ~7:00 P.M. and decreasing thereafter to reach a nadir of 1.2 ± 0.6 μmol · kg^{-1} · min^{-1} (0.2 ± 0.1 mg · kg^{-1} · min^{-1}) ~12 h later. HGP closely mirrored the changes in GIR, whereas G_{Rd} did not change significantly. Plasma FFA and cortisol levels paralleled HGP values, although the HGP nadir seemed to precede the cortisol nadir by several hours.

FFA, cortisol, growth hormone, glucagon, lactate, and alanine concentrations. Plasma FFA and cortisol concentrations changed with circadian rhythmicity with cycle dura-

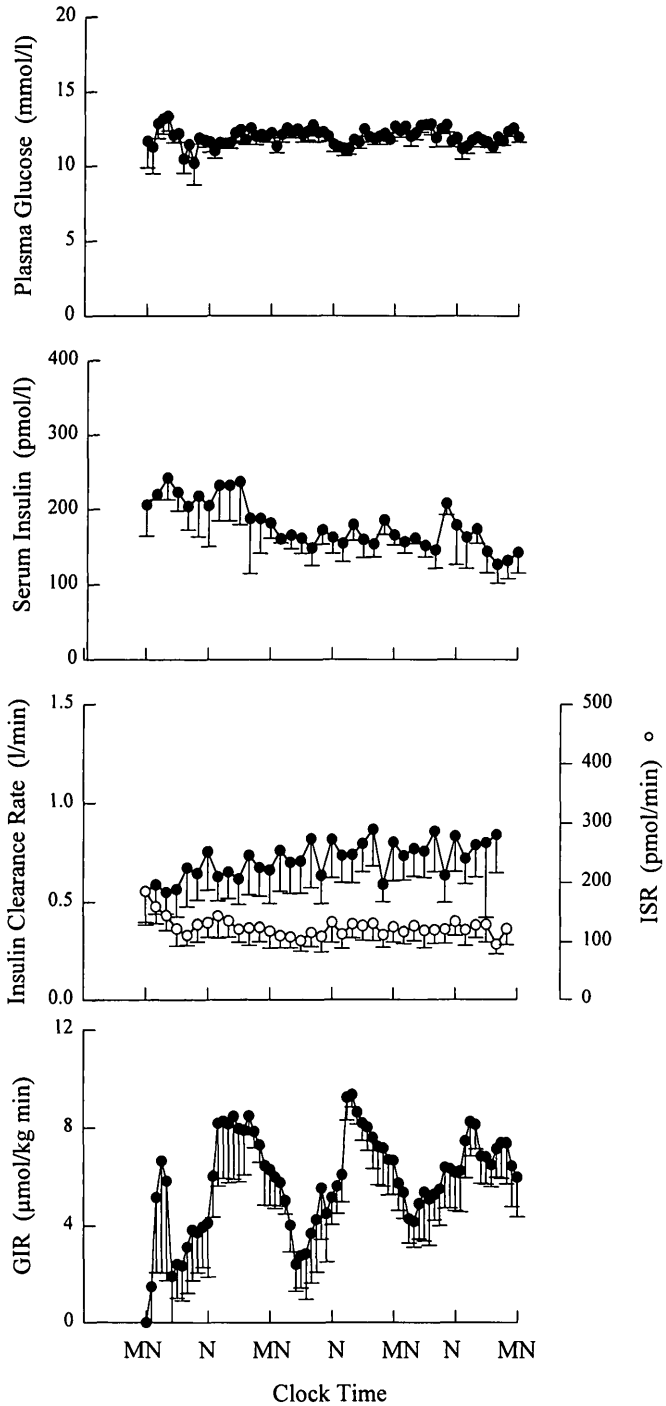

FIG. 1. Plasma glucose and serum insulin concentrations and ISR, insulin clearance rate, and GIR during 72 h of hyperglycemic clamping in six patients with NIDDM. (In one patient, the study lasted only 36 h.) MN, midnight; N, noon. Means ± SE are shown.

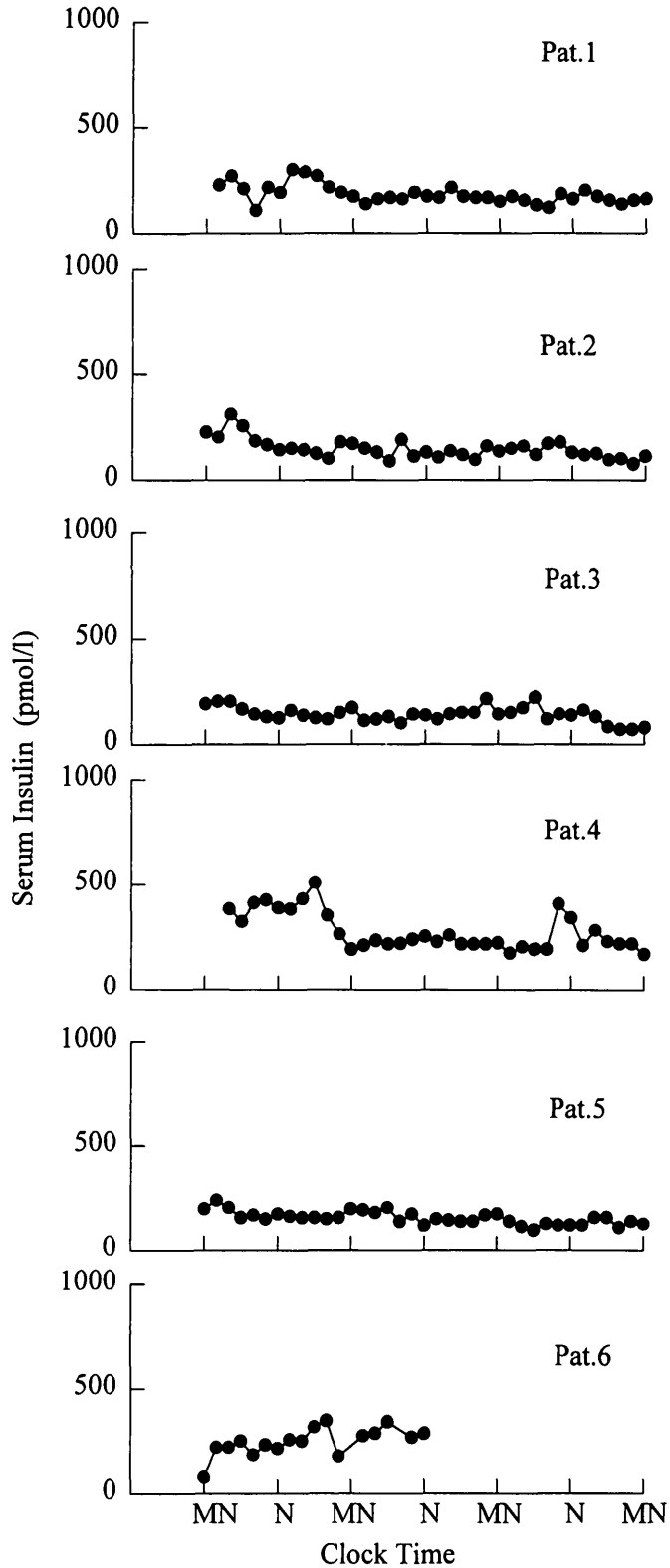

FIG. 2. Serum insulin concentrations during 72 h of hyperglycemic clamping in six patients with NIDDM. MN, midnight; N, noon. (In one patient, the study lasted only 36 h.)

tions of 24.3 ± 3.9 and 23.0 ± 1.3 h and amplitudes of 15.2 ± 5.9% (±97 μmol/l) and 41.8 ± 7.2% (±143 nmol/l), respectively. Changes in plasma FFA and cortisol concentration both correlated inversely with changes in GIR, i.e., both rose when GIR decreased and vice versa (Figs. 4 and 5). The correlations between plasma FFA concentrations and GIR and between plasma cortisol and GIR were highly significant ($r = -0.72$, $P < 0.001$, and -0.64, $P < 0.001$, respectively) (Fig. 6). In contrast, plasma growth hormone, glucagon, lactate, and alanine concentrations (Fig. 5) did not exhibit circadian changes and did not correlate with rates of GIR.

Polygraphic sleep analysis. Sleep was polygraphically monitored during one night of the 72-h study. Total recording time was 554 ± 22 min. Total sleep time (REM plus stages

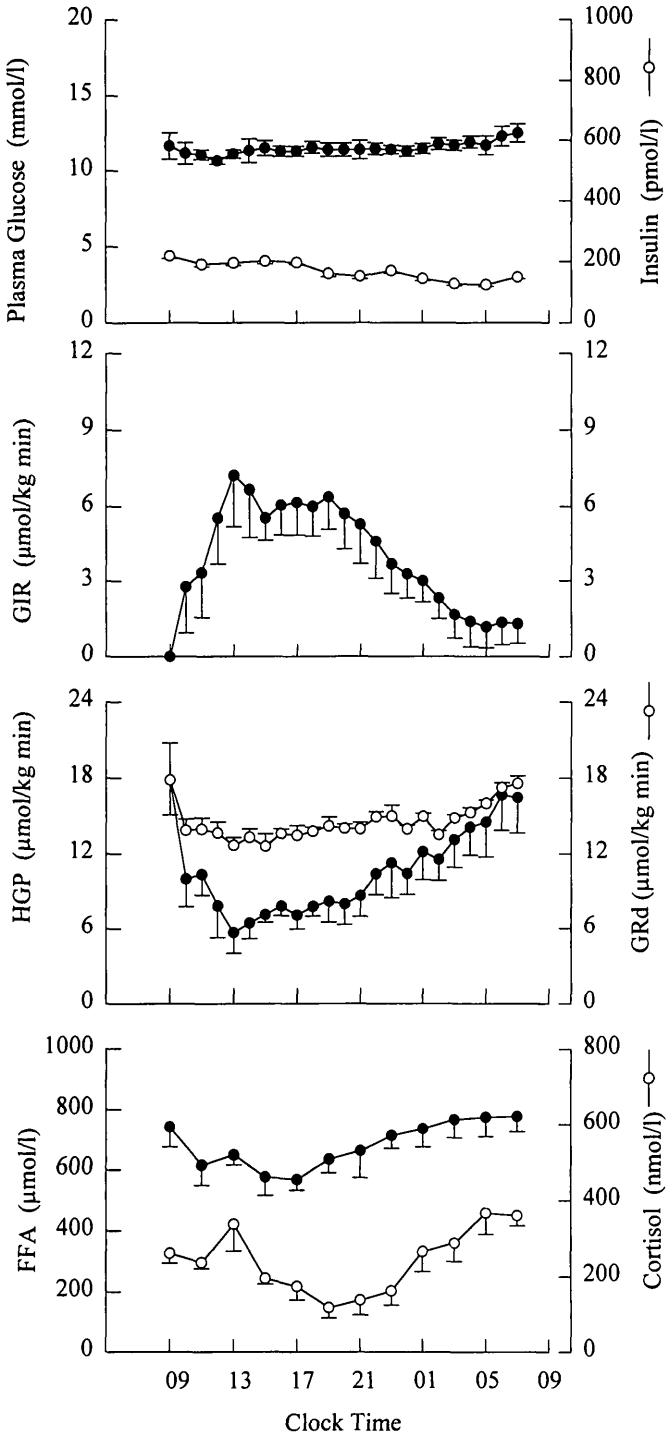

FIG. 3. Plasma glucose concentrations and GIR, HGP, G_{Rd}, and plasma FFA and cortisol levels during 24 h of hyperglycemic clamping in five patients with NIDDM.

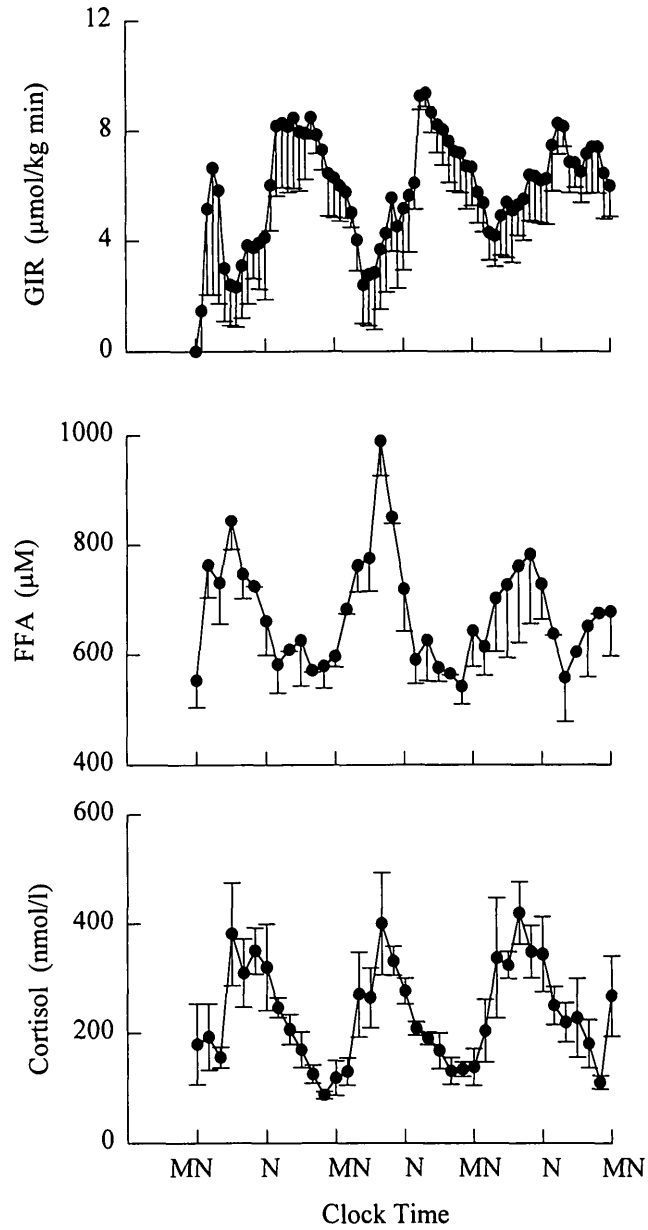

FIG. 4. GIR and plasma FFA and cortisol concentrations during hyperglycemic clamping for 72 h in six patients with NIDDM. MN, midnight; N, noon.

1–4) was 347 ± 25 min. Patients woke up 28 ± 6 times for a total wake time of 206 ± 36 min.

DISCUSSION

Absence of circadian rhythmicity of insulin secretion in NIDDM. We have recently reported that insulin secretion rates and serum insulin levels change with a circadian rhythm in nondiabetic subjects during hyperglycemic clamping (9). The absence of this rhythmicity is a newly described defect in patients with NIDDM. Absence of rhythmic insulin changes allowed us to study insulin sensitivity at constant blood glucose and insulin levels.

Presence of circadian rhythmicity of insulin sensitivity in NIDDM. Diurnal rhythmicity of insulin sensitivity and glucose tolerance in diabetic patients, although long suspected (1–5), has been difficult to demonstrate mainly because of the strong interdependence of changes in glucose and insulin and other confounding factors including feeding, exercise, and changes in insulin clearance (1). In the present study, the patients were kept on bed rest, they did not eat, and their blood glucose and insulin levels, and insulin secretion and clearance rates all remained unchanged during the entire 72-h study. GIR, reflecting total body insulin sensitivity, rose daily from a nadir in the morning (~8:00 a.m.) to a peak in the evening (~7:00 P.M.). The cycle duration (from peak to peak) was ~24 h hence; these cycles were by definition circadian. The cycle amplitude (from trough to peak) was ~7.8 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ (1.4 $mg \cdot kg^{-1} \cdot$

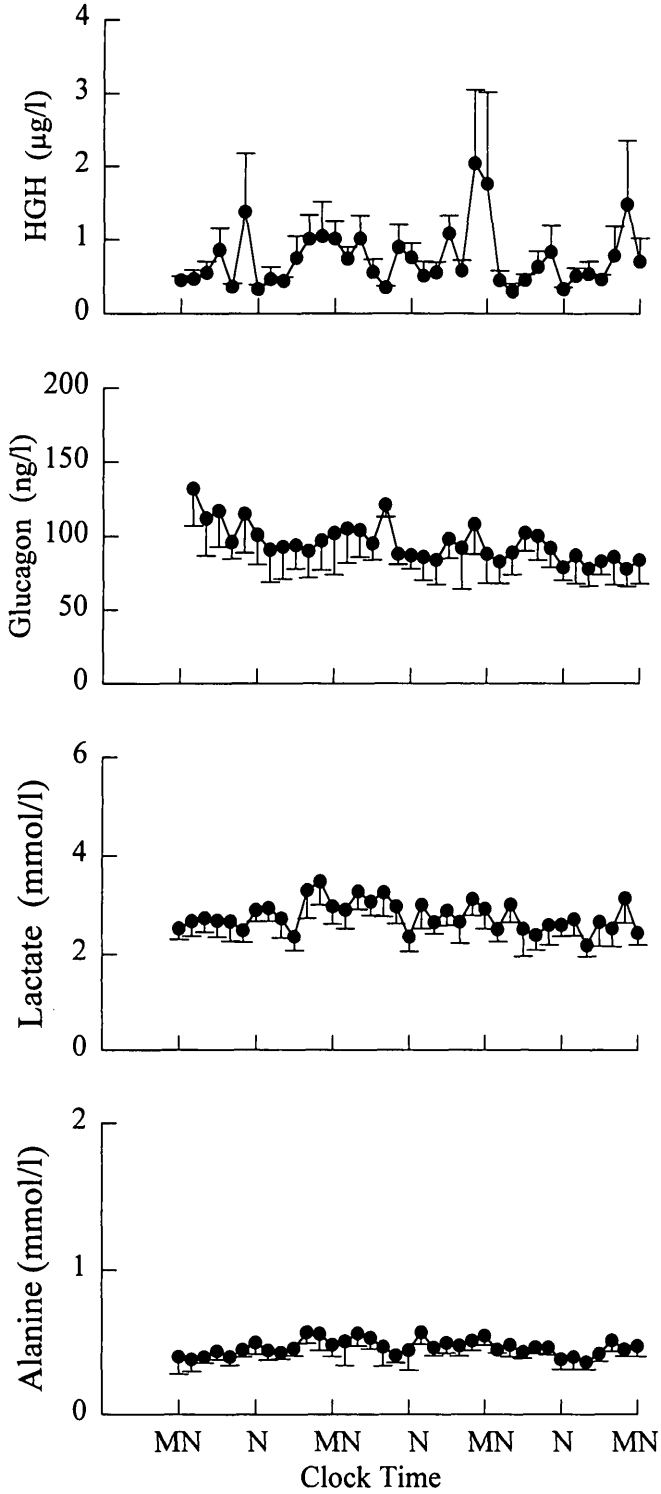

FIG. 5. Plasma growth hormone (HGH), glucagon, lactate, and alanine concentrations during hyperglycemic clamping for 72 h in six patients with NIDDM. MN, midnight; N, noon.

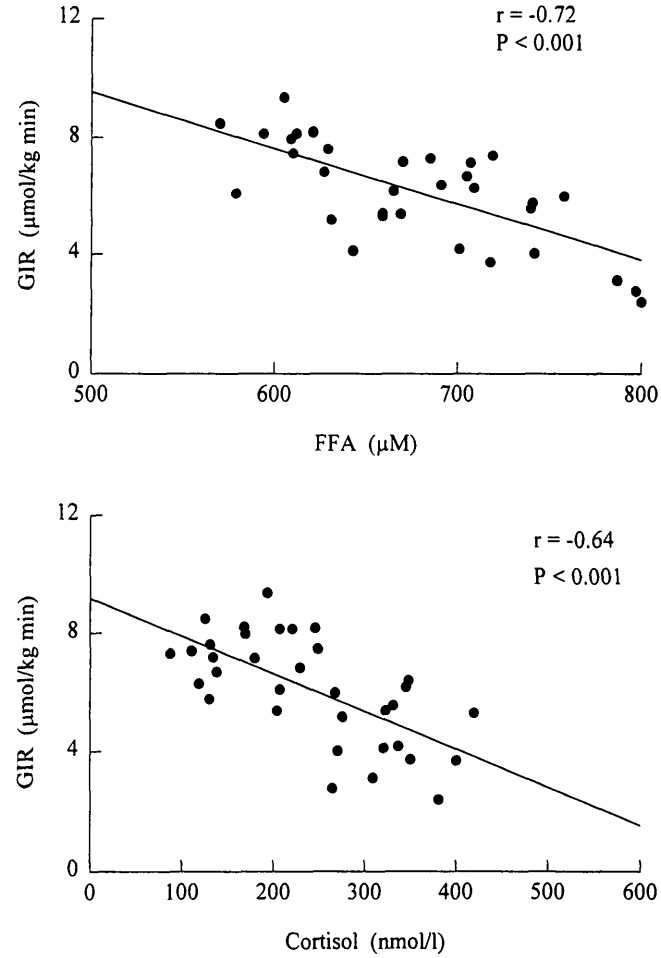

FIG. 6. Correlations (by least-squares regression analysis) between mean GIR and mean plasma FFA and cortisol concentrations during 72 h of hyperglycemic clamping in six patients with NIDDM.

min^{-1}). To our knowledge, this is the first unequivocal demonstration of circadian rhythmicity in insulin sensitivity in patients with NIDDM. Similar results have been obtained in normal volunteers in whom glucose was infused at constant rates. In these studies, glucose and insulin levels changed together, which complicated quantitation of cyclic changes in insulin sensitivity (19,20).

Sleep did not appear to affect the cyclic changes in insulin sensitivity because the directional changes (from increase to decrease and vice versa) occurred before and after the sleep period. Moreover, insulin sensitivity (GIR) was the same whether the patients were asleep or awake (4.7 ± 0.9 vs. 4.7 ± 0.6 μmol · kg^{-1} · min^{-1}). Sleep, however, was frequently interrupted; therefore, our data do not rule out the possibility that uninterrupted sleep may have a modulating effect on the circadian rhythmicity of insulin sensitivity as was reported by Van Cauter et al. in normal subjects (21).

What caused the circadian rhythmicity of insulin sensitivity? Changes in GIR could have reflected changes in peripheral (muscle) insulin sensitivity (i.e., in G_{Rd}), changes in hepatic insulin sensitivity (i.e., in HGP), or both. To differentiate between these possibilities, we determined G_{Rd} and HGP in five patients at hourly intervals for 22 h. Glucose was again clamped at ~11.6 mmol/l, and serum insulin levels remained stable. The results indicated unequivocally that the nocturnal decrease in GIR was caused by an increase in HGP with little or no change in peripheral glucose uptake. These results are compatible with a large body of evidence showing elevated rates of HGP in patients with NIDDM after an overnight fast (22) and with the demonstration that plasma glucose levels and HGP rates rise in the early morning hours (dawn phenomenon) (8,23). Our data suggested, however, that the dawn phenomenon may be explained, at least in part, by the circadian HGP cycle, where HGP starts to rise in

the evening, reaches a peak in the early morning hours, and then declines. Superimposed on this circadian rhythm may be the effects of sleep on HGP, because Clore et al. (24) have shown in normal subjects that sleep decreased and arousal from sleep increased HGP.

What caused the circadian HGP changes? Plasma FFA concentrations showed circadian fluctuations and correlated inversely ($r = -0.72$, $P < 0.001$) with GIR (and presumably with HGP). Similar close relationships between plasma FFA and HGP have recently been demonstrated in normal volunteers (24) and in dogs (25). These studies suggested that FFAs stimulated HGP, probably by changing hepatic insulin sensitivity. While this concept has been well supported by many in vitro studies (26), the in vivo evidence remains controversial. Elevation of plasma FFA concentrations has been shown to increase HGP only when fatty acid–mediated stimulation of insulin secretion was prevented (27). In addition, several studies have shown that acute elevation of plasma FFAs resulted in the inhibition of insulin-induced suppression of HGP (28,29). It was not clear, however, whether this effect was produced by elevated FFA levels or by the glycerol that was present in the commercial triglyceride emulsions infused in these studies (29). The present study was not complicated by either of these confounders. The results thus support the notion that FFAs can produce hepatic as well as peripheral insulin resistance (29,30). In this respect, it needs to be emphasized that FFAs have been shown to inhibit only insulin-stimulated glucose uptake but not basal glucose uptake (29). The present study was performed under isoglycemic, i.e., basal conditions; hence, FFA did not inhibit glucose uptake.

Plasma cortisol, which is known to fluctuate in circadian cycles, also correlated negatively with GIR ($r = -0.64$, $P < 0.001$). In patients with IDDM, physiological elevations in plasma cortisol have been shown to markedly increase HGP (31) and, to a lesser degree, to cause peripheral insulin resistance (32,33). Recent studies in adrenalectomized rats indicated that glucocorticoids played a key role in the fatty acid-induced stimulation of gluconeogenesis (34). This and data from normal human subjects showing that isolated cortisol deficiency reduced plasma FFA concentrations by ~50% raised the interesting possibility that circadian cortisol secretion may have been, at least partly, responsible for the circadian FFA rhythmicity (35). Clearly, however, our results did not establish a cause and effect relationship between FFAs, cortisol, and HGP. In fact, analysis of individual relationships showed that in three of the five patients, plasma cortisol and HGP changed in synchrony, while in two patients cortisol started to rise several hours after HGP. These findings do not support the notion that cortisol was primarily responsible for the rhythmic changes in HGP, because it has been shown that the cortisol effects on HGP required several hours to develop (31,33).

Growth hormone levels increased at the onset of sleep. There was, however, no circadian pattern nor did growth hormone levels correlate with GIR. Nevertheless, some growth hormone bursts may have been missed because of the 2-h blood sampling, and the evening growth peaks may have contributed to the nocturnal rise in HGP. Plasma glucagon concentrations did not change significantly. Epinephrine, which is known to have a circadian rhythm of secretion (36), was not measured. The amplitude of the circadian epinephrine changes, however, are known to be too small to affect HGP directly (32,37), but they may be able to affect lipolysis and thus plasma FFA concentrations.

Clinical significance. The circadian rhythmicity of insulin sensitivity in patients with NIDDM, described here, predicts that glucose tolerance will be worse in the early morning than at any other time. This helps to explain why insulin requirements in diabetic patients are highest in the morning. It also provides a rationale for the effectiveness of the twice-a-day regimen, which consists of bedtime insulin and daytime sulfonylurea administration, the bedtime insulin acting to prevent the nocturnal rise in HGP. Lastly, our data suggested that suppression of the nocturnal FFA and/or cortisol peaks may offer a new approach to the control of fasting blood glucose levels in patients with NIDDM.

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