Measurement of Plasma Glucose, Free Fatty Acid, Lactate, and Insulin for 24 h in Patients With NIDDM

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Fasting and postprandial plasma glucose, free fatty acid (FFA), lactate, and insulin concentrations were measured at hourly intervals for 24 h in 27 nonobese individuals—9 with normal glucose tolerance, 9 with mild non-insulin-dependent diabetes mellitus (NIDDM, fasting plasma glucose <175 mg/dl), and 9 with severe NIDDM (fasting plasma glucose >250 mg/dl). In addition, hepatic glucose production (HGP) was measured from midnight to 0800 in normal individuals and patients with severe NIDDM. Plasma glucose concentration was highest in patients with severe NIDDM, lowest in those with normal glucose tolerance, and intermediate in those with mild NIDDM (two-way ANOVA, $P < .001$). Variations in plasma FFA and lactate levels of the three groups were qualitatively similar, with lowest concentrations seen in normal individuals, intermediate levels in the group with mild NIDDM, and the highest concentration in those with severe NIDDM (two-way ANOVA, $P < .001$). Of particular interest was the observation that plasma FFA concentrations were dramatically elevated from midnight to 0800 in patients with severe NIDDM. The 24-h insulin response was significantly increased in patients with mild NIDDM, with comparable values seen in the other two groups. Values for HGP fell progressively throughout the night in normal individuals and patients with severe NIDDM, despite a concomitant decline in plasma glucose and insulin levels. Although the magnitude of the fall in HGP was greater in NIDDM, the absolute value was significantly ($P < .001$) greater than normal throughout the period of observation. These results demonstrate that there are differences in substrate level between individuals with normal glucose tolerance and patients with NIDDM and differing degrees of glucose intolerance, unrelated to ambient insulin level, and these changes persist over 24 h. *Diabetes* 37:1020–24, 1988

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Our laboratory has recently published results documenting that circulating plasma free fatty acid (FFA) concentrations are elevated in patients with non-insulin-dependent diabetes mellitus (NIDDM) and suggested that this change plays a major role in the development of fasting hyperglycemia (1–4). We proposed that the increase in plasma FFA concentration stimulated hepatic glucose production (HGP), leading to fasting hyperglycemia in patients characterized by a defect in insulin-stimulated glucose uptake (4). Although this formulation was consistent with available data, we realized that major issues remained to be addressed. For example, our view that elevated FFA levels are responsible for fasting hyperglycemia was based on measurements of plasma FFA concentration before and for 4 h after breakfast and lunch. As a result, we realized we had essentially no information concerning FFA metabolism throughout most of the 24-h period. In addition, although elevated FFA levels may stimulate HGP (5,6), they cannot serve as a substrate for glucose. Therefore, speculation that an increase in ambient plasma FFA concentration stimulated HGP required identification of a glucogenic precursor to help fulfill this function. An obvious contender for this role is lactate, and we were unaware of experimental data documenting the relationship between plasma lactate, FFA, and glucose concentrations in patients with NIDDM. Consequently, we decided to initiate this investigation in which the period of observation was radically enlarged, starting at 0800, and involved hourly measurement of plasma glucose, insulin, FFA, and lactate concentrations for 24 h consecutively, both before and after meals.

MATERIALS AND METHODS

The study population consisted of 27 nonobese individuals—9 with normal glucose tolerance and 18 with NIDDM (7). The patients with NIDDM were further subdivided into

TABLE 1
Clinical characteristics of study population

Group	*n* (M/F)	Age (yr)	Body mass index (kg/m²)
Normal	6/3	52 ± 5	26.8 ± 0.7
Mild NIDDM	5/4	54 ± 3	27.5 ± 1.3
Severe NIDDM	5/4	54 ± 4	27.3 ± 1.2

Values are means ± SE.

two groups, designated as having severe (fasting plasma glucose >250 mg/dl) or mild (fasting plasma glucose <175 mg/dl) diabetes. Degree of obesity was estimated by use of the body mass index (BMI = kg/m²), and patients were classified as being nonobese if their BMI was <30 kg/m² (8). None of the patients with NIDDM had ever taken insulin, and those taking sulfonylureas had their medication discontinued for at least 1 mo before these studies. All subjects were in good health, and none was taking any drug known to affect carbohydrate metabolism. Some relevant clinical characteristics of the three groups are shown in Table 1.

This study was approved by the Stanford University Human Subjects Committee, and each individual signed a consent form when admitted to the Stanford General Clinical Research Center. All volunteers were fed an isocaloric (35 cal/kg) diet containing (as percentage of total calories) 17% protein, 40% fat, and 43% carbohydrate, and each meal had this relative content of nutrients. Meals, which were eaten at 0800, noon, and 1800, contained 20, 40, and 40%, respectively, of the day's total calorie intake. The experimental measurements to be described subsequently were initiated after at least 3 days of dietary stabilization.

Experimental measurements. To assess ambient plasma hormone and substrate concentrations, measurements were made of plasma glucose, FFA, lactate, and insulin concentrations, both in the fasting state and in response to breakfast (0800), lunch (noon), and dinner (1800). Blood was drawn before breakfast at 0800, and then at hourly intervals for the next 24 h. Plasma was separated immediately, frozen, and subsequently analyzed for plasma glucose (9), FFA (10), lactate (11), and insulin (12) concentrations. All samples for each individual were analyzed at the same time, and each assay contained samples from each of the three groups. In addition, glucose appearance rate (R_a) was determined from midnight until 0800 in six of the nine normal individuals and eight of the nine patients with severe NIDDM. Each subject received a bolus of 30 μCi i.v. [3-³H]glucose at 0800, followed by a continuous infusion of tracer at a rate of 0.3 μCi/min for the next 12 h. Aliquots of plasma obtained at 30-min intervals were either analyzed for plasma glucose concentration (9) or precipitated with $Ba(OH)_2$ and $ZnSO_4$, and glucose and radioactivity were determined in the protein-free supernatant. Glucose specific activity was then determined, and R_a was calculated with the non-steady-state equation of Steele (13). In these studies, values of R_a were generated hourly, beginning at midnight, with the data from the last 30 min preceding these times used for the calculations. Because these studies were carried out under fasting conditions, R_a was assessed to be equal to the hepatic glucose production (HGP) rate.

Statistical analysis. Data are expressed as means ± SE and were analyzed by the statistical analysis system with the general linear-models procedure. To evaluate differences between nondiabetic and diabetic individuals over time, data were analyzed by two-way analysis of variance (ANOVA; 14,15). For this analysis, the two dependent variables were time (hour of the day) and group (normal vs. NIDDM).

RESULTS

Plasma glucose concentrations throughout the 24-h observation are seen in Fig. 1. It is apparent that plasma glucose

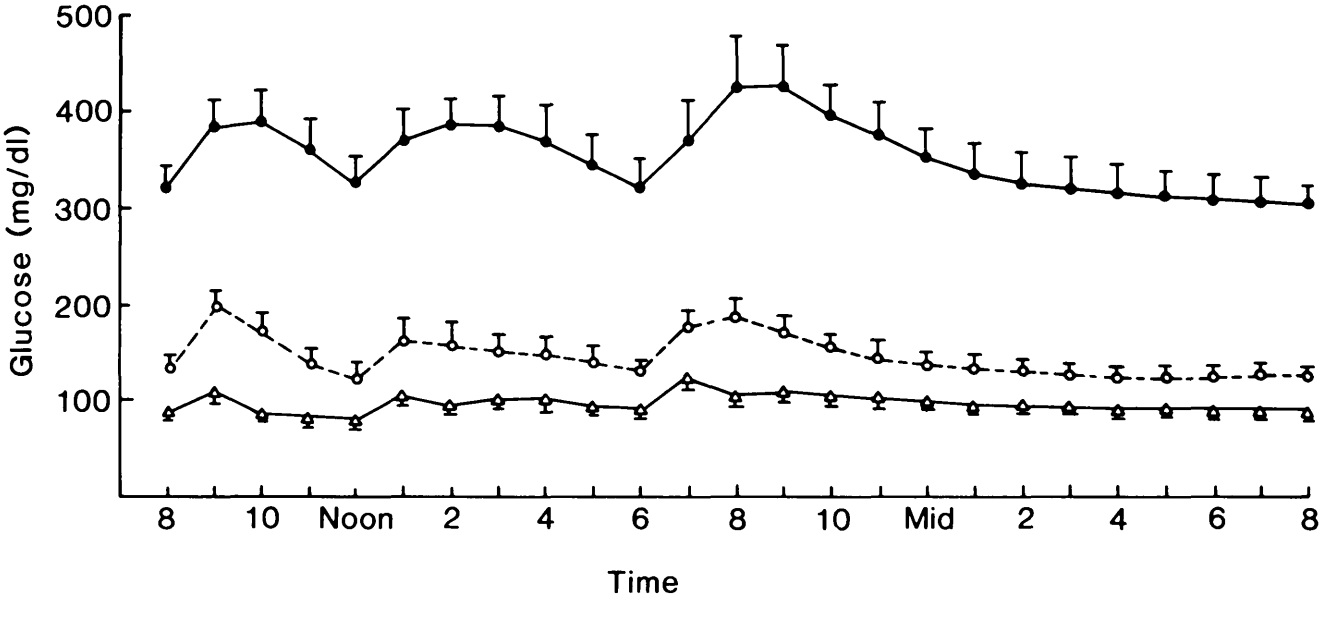

FIG. 1. Mean ± SE plasma glucose concentration measured at hourly intervals for 24 h. Meals were eaten at 0800 (20% of total calories), noon (40% of total calories), and 1800 (40% of total calories) in normal (△), mildly non-insulin-dependent diabetic (fasting plasma glucose <175 mg/dl, ○), and severely non-insulin-dependent diabetic (fasting plasma glucose >250 mg/dl, ●) individuals.

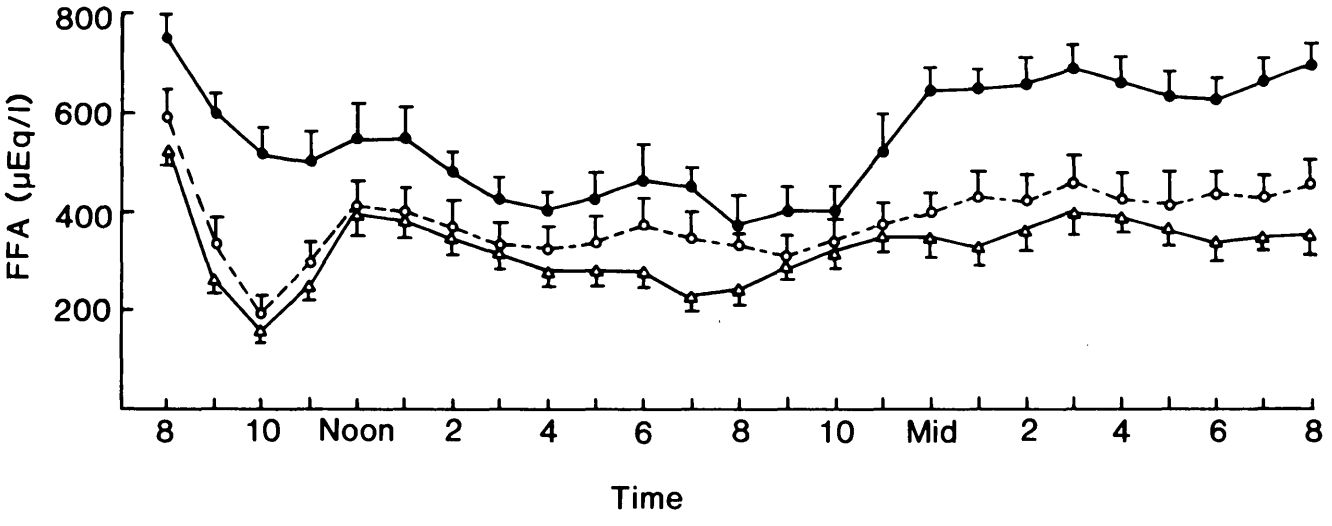

FIG. 2. Mean ± SE free fatty acid (FFA) concentration measured at hourly intervals for 24 h. Meals were eaten at 0800 (20% of total calories), noon (40% of total calories), and 1800 (40% of total calories) in normal (△), mildly non-insulin-dependent diabetic (○), and severely non-insulin-dependent diabetic (●) individuals.

concentrations were higher than control values in patients with NIDDM, and this was true of both groups ($P < .001$). In addition, the 24-h plasma glucose response was significantly ($P < .001$) greater in patients with severe compared with mild NIDDM.

Plasma FFA concentrations throughout the 24-h period are displayed in Fig. 2. These results indicate that FFA levels were higher in the group with severe diabetes than in either of the other two groups ($P < .001$). The difference was particularly dramatic from approximately midnight to 0800 the next morning, with plasma FFA concentrations being almost twice as high in individuals with severe diabetes. Note that the 24-h FFA response of patients with mild NIDDM was also significantly ($P < .001$) greater than in control subjects. However, plasma FFA values in the two groups were similar from 0800 to 1500.

Plasma lactate concentrations for the 24 h of observation are shown in Fig. 3. Lactate levels increased after each meal, demonstrating the same general profile as the plasma glucose responses. Thus, the plasma lactate response over the 24-h period was significantly ($P < .001$) increased over the control response in both groups of patients with NIDDM. In addition, the 24-h lactate response of the patients with severe diabetes was greater than that of the mildly diabetic patients ($P < .001$).

Plasma insulin concentrations are shown in Fig. 4. The 24-h plasma insulin response was significantly ($P < .001$) greater in the patients with mild diabetes than in the other two groups. Although the plasma insulin response was somewhat lower than control values in patients with severe diabetes, the difference between the two groups over the 24-h period was not statistically significant ($P = .37$). However, plasma insulin levels were significantly lower in patients with severe NIDDM when only values from 0800 to 2000 were considered.

Values for HGP in normal individuals and patients with severe NIDDM are seen in Fig. 5. The data displayed represent measurements of HGP at 2-h intervals from midnight to 0800 and indicate that HGP was significantly ($P < .001$) elevated throughout this period in patients with NIDDM. However, note that the magnitude of the difference between the two groups declined progressively with time, falling from

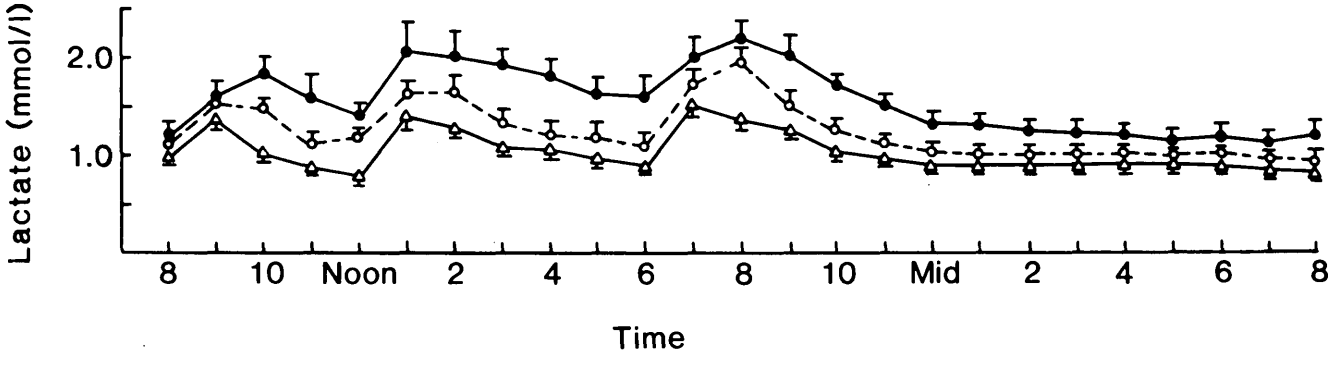

FIG. 3. Mean ± SE lactate concentration measured at hourly intervals for 24 h. Meals were eaten at 0800 (20% of total calories), noon (40% of total calories), and 1800 (40% of total calories) in normal (△), mildly non-insulin-dependent diabetic (○), and severely non-insulin-dependent diabetic (●) individuals.

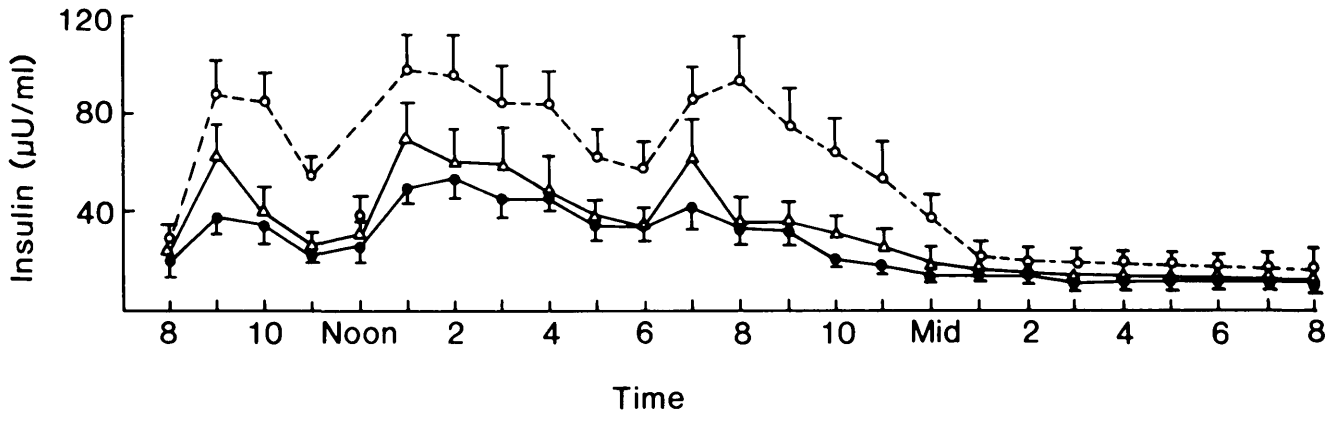

FIG. 4. Mean ± SE insulin concentration measured at hourly intervals for 24 h. Meals were eaten at 0800 (20% of total calories), noon (40% of total calories), and 1800 (40% of total calories) in normal (△), mildly non-insulin-dependent diabetic (○), and severely non-insulin-dependent diabetic (●) individuals.

~1.0 mg · kg^{-1} · min^{-1} at midnight to 0.5 mg · kg^{-1} · min^{-1} at 0800. Also, the primary reason for the narrowing of the gap in values for R_a is that R_a fell by 34% in patients with NIDDM from midnight until 0800.

DISCUSSION

The results of these studies can be discussed on several levels. Generally, they emphasize that perception of biological phenomena can vary substantially as a function of experimental protocol. This principle is most dramatically apparent in Fig. 2. These data show that plasma FFA concentrations were higher on the average in patients with severe NIDDM throughout the 24-h observation period. However, the magnitude of the difference between the groups was much greater over the last 8 h, when the levels were almost twice as high in those with severe diabetes than in the other two groups. Obviously, an estimate of the quantitative nature of the defect in insulin's ability to regulate plasma FFA concentration would vary substantially as a function of the period examined.

Our results are also extremely relevant to the question regarding the relationship between circulating plasma FFA and lactate levels in regulation of HGP. The results in Fig. 5 confirm previous observations that values for HGP are greater than normal in patients with NIDDM (4;16–18). However, when measurements were made continuously throughout the night, new insights into this general phenomenon evolved. Although it is evident that HGP fell progressively from midnight to 0800 in both normal individuals and in patients with NIDDM and severe fasting hyperglycemia, the magnitude of the fall was greater in patients with NIDDM, resulting in a progressive narrowing of the difference in HGP values between the two groups. Note that the progressive fall in HGP from midnight to 0800 took place despite a concomitant fall in both plasma glucose and insulin concentration. Thus, there are two questions that must be addressed to explain these results. Generally, it is necessary to define the factor(s) responsible for the suppression of HGP when both plasma glucose and insulin levels are declining. Unfortunately, our study was not designed to address this issue, and an unequivocal answer cannot be generated from the data. It can be speculated, however, that it is a reduced rate of substrate flow from peripheral tissue to the liver after the last meal that may account for the fall in HGP. The fact that

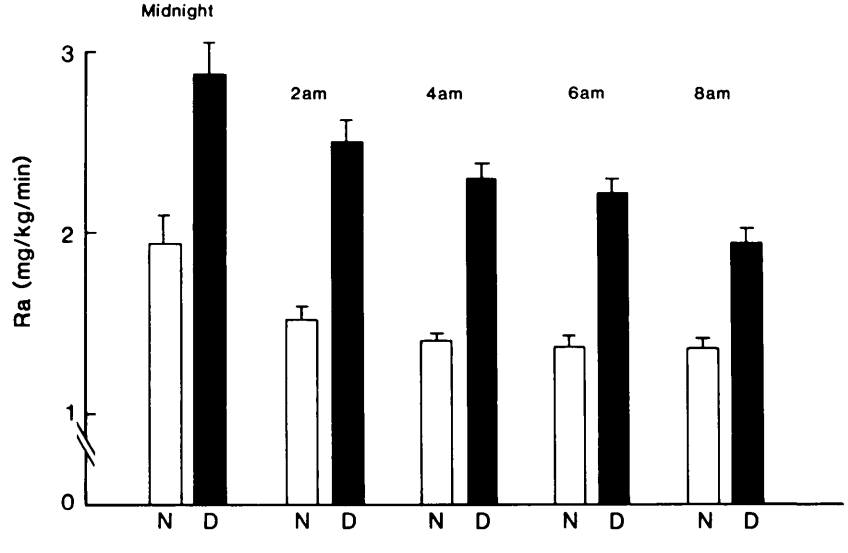

FIG. 5. Mean ± SE values for glucose appearance rate (R_a) measured at 2-h intervals from midnight to 0800 in 6 normal individuals (□) and 8 patients with severe non-insulin-dependent diabetes (■).

a fall in plasma lactate concentration from a peak value 2 h after dinner antedated the decline in R_a supports this possibility.

The second issue that must be addressed is why HGP is higher in patients with NIDDM. An obvious answer is that the ability of plasma glucose and/or insulin to inhibit HGP is defective in patients with severe NIDDM. Indeed, decreased insulin activity in patients with severe NIDDM may have been responsible for all the changes noted, i.e., elevated plasma glucose, lactate, and FFA concentrations and the increase in HGP. However, the elevated plasma FFA levels in the patients with severe NIDDM might have contributed to the difference in HGP noted in Fig. 5. We have previously documented a relationship between the increment in plasma glucose concentration, HGP, and ambient FFA levels in patients with NIDDM (4). Our results highlight the considerable magnitude of the elevation in plasma FFA levels that occurs between midnight and 0800 in patients with severe NIDDM. It is not unreasonable to speculate that FFA oxidation by both liver and muscle would increase, leading to a decrease in hepatic and muscle pyruvate dehydrogenase activity (19,20). The predicted result of this change in enzyme activity would be an increase in the conversion of glucose to lactate in muscle, enhanced hepatic gluconeogenesis, and severe hyperglycemia. Thus, the changes in plasma FFA and lactate noted over the 24-h study period in the patients with severe NIDDM are consistent with the view that elevated plasma FFA and lactate levels play an important role in the development of fasting hyperglycemia in patients with NIDDM.

Because the magnitude of the difference in plasma lactate levels between the two groups of diabetic subjects was not as extreme as the difference in FFA concentrations, it could be argued that the defect in FFA metabolism is most essential for the development of severe fasting hyperglycemia. On the other hand, we measured plasma concentration, not turnover, which may have led to an underestimate of the change in lactate metabolism. Furthermore, stimulation of HGP by FFA and/or lactate need not be linearly related to either the concentration or the turnover rate of the two substrates. Thus, it seems safest to simply conclude by indicating that measurements of plasma FFA and lactate concentration over 24 h demonstrate that plasma insulin levels, which are similar to those of normal individuals in absolute terms, are not capable of maintaining normal plasma FFA and lactate levels. These results provide support for the view that increased HGP and severe hyperglycemia in NIDDM may be related to the defect in insulin regulation of FFA and lactate metabolism.

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