Evidence of Increased Systemic Glucose Production and Gluconeogenesis in an Early Stage of NIDDM

Gabriele Perriello, Simone Pampanelli, Paola Del Sindaco, Carlo Lalli, Marco Ciofetta, Elena Volpi, Fausto Santeusanio, Paolo Brunetti, and Geremia B. Bolli

To assess the mechanisms of fasting hyperglycemia in NIDDM patients with mild elevation of fasting plasma glucose (FPG) compared with NIDDM patients with overt hyperglycemia, we studied 29 patients with NIDDM, who were divided in two groups according to their fasting plasma glucose (<7.8 and ≥7.8 mmol/l for groups A and B, respectively), and 16 control subjects who were matched with NIDDM patients for age, sex, and body mass index. All subjects were infused with [3-^{3}H]glucose between 10:00 P.M. and 10:00 A.M. during overnight fasting to determine glucose fluxes. In 27 subjects (17 diabetic and 10 control), [U-^{14}C]alanine was simultaneously infused between 4:00 A.M. and 10:00 A.M. to measure gluconeogenesis (GNG) from alanine. Arterialized-venous plasma samples were collected every 30 min for measurement of glucose fluxes, GNG, and glucoregulatory hormones. In group A, plasma glucose, rate of systemic glucose production (SGP), and GNG were greater than in control subjects (7.2 ± 0.2 vs. 4.9 ± 0.1 mmol/l, 10.9 ± 0.2 vs. 9.5 ± 0.3 μmol · kg^{-1} · min^{-1}, and 0.58 ± 0.04 vs. 0.37 ± 0.02 μmol · kg^{-1} · min^{-1}, respectively, for group A and control subjects; mean value 8:00 A.M.–10:00 A.M., all $P < 0.05$). Both increased SGP and GNG correlated with plasma glucose in all subjects ($r = 0.77$ and $r = 0.75$, respectively, $P < 0.005$). Plasma counterregulatory hormones did not differ in NIDDM patients compared to control subjects. The present studies demonstrate that SGP and GNG are increased in NIDDM patients without overt fasting hyperglycemia. Thus these metabolic abnormalities primarily contribute to early development of overnight and fasting hyperglycemia in NIDDM. *Diabetes* 46:1010–1016, 1997

In patients with NIDDM, the mechanisms of fasting hyperglycemia have been largely investigated in the past decade (1–7). Most (1–5) but not all studies (6,7) have demonstrated that hepatic glucose production is greater than normal in NIDDM (1–5) and correlates with fasting hyperglycemia (2,4). Nowadays, however, the term "systemic glucose production" (SGP) should replace the more popular term "hepatic glucose production" because there is now solid evidence that the kidney, as well as the liver, is an important source of glucose production in humans (8).

Although it is now widely accepted that SGP is elevated in clinically overt NIDDM (9–11), whether SGP is also increased in NIDDM with only mild increases in fasting plasma glucose (FPG) is unclear (12,13). For example, in four studies (13–16), SGP in NIDDM with FPG <7.8 mmol/l was found to be similar to that of nondiabetic subjects, at least in absolute terms; however, in another study (12), the opposite conclusión was reached. Among other problems, one may have been an insufficient power to detect statistical increases in fasting SGP with mild increases in FPG between subjects with NIDDM and nondiabetic individuals.

To explain the development of hyperglycemia in the early stages of NIDDM, it has been suggested that an impaired peripheral glucose uptake, not an augmented SGP, is primarily responsible for initiation of hyperglycemia (14). According to this view, the increase in SGP is a late event in the natural history of NIDDM, and it appears to be determined by increased availability and oxidation of plasma free fatty acids (17). This hypothesis has been supported by the observation that in NIDDM, despite normalization of SGP after 20 h of fasting, hyperglycemia remains (18). However, the "normal" rates of SGP in that study (18) might be interpreted as "abnormal" in view of an approximately threefold greater than normal prevailing plasma glucose concentration.

More recently, Jeng et al. (19) found that, in NIDDM patients with different degrees of hyperglycemia, SGP is not greater than normal until FPG is >10 mmol/l. On the other hand, a direct correlation between SGP and FPG from 6 to 8 mmol/l has been observed (12), suggesting that SGP may be increased in NIDDM patients with FPG <7.8 mmol/l as well. Thus the mechanism(s) of fasting hyperglycemia in NIDDM with mild elevation of FPG (i.e., <7.8 mmol/l) remains controversial.

The present studies were undertaken to assess whether the rates of SGP are elevated in NIDDM patients with FPG <7.8 mmol/l and BMI of 26.2 ± 0.6 compared with NIDDM patients with overt fasting hyperglycemia and BMI of 27.8 ± 0.5. For this purpose, 29 NIDDM patients were infused with [3-^{3}H]glucose overnight to determine glucose fluxes. Of these, 17 were simultaneously infused with [U-^{14}C]alanine to measure gluconeogenesis (GNG) from alanine. To the best of our knowledge, the latter information has never been obtained in NIDDM with mild fasting hyperglycemia, but only in NIDDM patients with clinically overt hyperglycemia (20,21).

RESEARCH DESIGN AND METHODS

Subjects. After being approved by the local Institutional Review Board, 45 individuals (29 with NIDDM and 16 control subjects) participated in these studies. NIDDM subjects were divided into two groups according to their FPG: group A,

From the Dipartimento di Medicina Interna e Scienze Endocrine e Metaboliche,Università di Perugia, I-06126 Perugia, Italy.

Address correspondence and reprint requests to Geremia B. Bolli, MD, Dipartimento di Medicina Interna e Scienze Endocrine e Metaboliche, Via E Dal Pozzo, I-06126 Perugia, Italy.

Received for publication 9 September 1996 and accepted in revised form 4 February 1997.

FPG, fasting plasma glucose; GNG, gluconeogenesis; SGP, systemic glucose production.

TABLE 1
Clinical characteristics of subjects studied

	Control	NIDDM group A	NIDDM group B
n	16	15	14
Men/women	11/5	11/4	6/8
Age (years)	51 ± 3	52 ± 2	54 ± 2
Body weight (kg)	73 ± 2	73 ± 2	79 ± 3
BMI (kg/m^2)	26.2 ± 0.4	26.2 ± 0.6	27.8 ± 0.5
Diabetes duration (years)	—	3.1 ± 0.5	5.6 ± 0.9*
Fasting plasma glucose (mmol/l)	4.9 ± 0.1	7.0 ± 0.2†	12.3 ±0.3*†
HbA_{1c} (%)	4.9 ± 0.2	6.7 ± 0.2†	10.2 ± 0.3*†
Fasting plasma insulin (pmol/l)	48 ± 3	76 ± 3†	57 ± 3*

Data are means ± SE. In control subjects, HbA_{1c} ranged from 3.8 to 5.5%. *$P < 0.05$ vs. NIDDM group A. †$P < 0.05$ vs. control.

with FPG <7.8 mmol/l (7.0 ± 0.2 [SE] mmol/l; n = 15 [11 men, 4 women]), and group B, with FPG ≥7.8 mmol/l (12.3 ± 0.3 mmol/l; n = 14 [6 men, 8 women]). Among the patients of group B, five were on sulfonylurea treatment, and one was on combined metformin-sulfonylurea therapy. In these patients, antihyperglycemia treatments were withdrawn at least 1 week before the study. Neither NIDDM patients nor nondiabetic subjects were obese (22). The clinical characteristics of NIDDM and control subjects are shown in Table 1.

NIDDM subjects were recruited from patients attending the outpatient clinic of the Department of Internal Medicine and Endocrine and Metabolic Sciences of the University of Perugia. Subjects with FPG of 6–8 mmol/l and control subjects matched for age (51 ± 3 years), sex (11 men, 5 women), and BMI (26.2 ± 0.4) had undergone an oral glucose tolerance test and were either diagnosed as having or excluded from having NIDDM, respectively, according to conventional criteria (23). Subjects with impaired glucose tolerance were not included in the study. The diabetic subjects were then treated only with a standard diet. Apart from diabetes, they had no diseases and were not taking any medication.

Protocol. After giving informed consent, all subjects were admitted to the clinical research unit of the Department of Internal Medicine and Endocrine and Metabolic Sciences between 4:00 and 5:00 P.M. and consumed their usual evening meal (10 kcal/kg). Starting at 9:00 P.M., they rested in bed and maintained a supine position throughout the experiments. To obtain arterialized-venous blood samples (24), a hand vein was cannulated in retrograde fashion with a 21-gauge butterfly needle and maintained at 65°C in a thermoregulated Plexiglas box. At 10:00 P.M., an antecubital vein of the contralateral arm was cannulated with an 18-gauge catheter and used for primed (proportional to plasma glucose concentrations at a ratio of 25 μCi:5 mmol/l plasma glucose) continuous (0.25 μCi/min) infusion of [3-^{3}H]glucose (Amersham, Arlington Heights, IL) for isotopic determination of systemic glucose production. In 17 NIDDM patients (9 from group A, 8 from group B) and 10 control subjects, [U-^{14}C]alanine (20 μCi, 0.20 μCi/min; Amersham) was simultaneously infused from 4:00 to 10:00 A.M. to determine rates of GNG from alanine. In total, 4 h were allowed for isotopic equilibration. Blood samples were drawn from the heated vein every 30 min from 2:00 until 10:00 A.M. for measurement of plasma glucose concentration and specific activity and hormones and from 8:00 until 10:00 A.M. for determination of alanine concentration and specific activity in the 27 subjects infused with [U-^{14}C]alanine. All subjects slept between midnight and 7:00 A.M.

Analytic procedures. All blood samples were placed on ice and centrifuged within 30 min at 4°C. Plasma samples were stored at –20°C before measurements.

Plasma glucose was determined with a glucose analyzer (Beckman Glucose Analyzer II, Beckman Instruments, Fullerton, CA). Plasma alanine concentration was determined by spectrofluorometry (25). Plasma [^{3}H]- and [^{14}C]glucose and [^{14}C]alanine specific activities were determined by ion-exchange chromatography, after deproteinization with zinc sulfate and barium hydroxide, as previously reported (26). Plasma insulin and counterregulatory hormones were measured according to previously described methods (27,28). HbA_{1c} was determined by high-performance liquid chromatography (29). In our laboratory, the range of HbA_{1c} in nondiabetic subjects is 3.8–5.5%.

Plasma [^{14}C]alanine specific activity was determined on the eluate from the AG-50 cation-exchange column by high-performance liquid chromatography, as previously described (30). Briefly, the eluate was dried and resuspended in 200 μl of 0.05 mol/l Na acetate, filtered, and injected into a C_{18} reverse-phase column after *o*-phthaldialdehyde derivatization.

Calculations. Glucose production and utilization were calculated by using the non-steady-state equation of De Bodo et al. (30a) and were smoothed according to the method of Miles et al. (30b), as previously reported (31). In the 27 subjects (17 NIDDM patients and 10 control subjects) who were infused simultaneously with [3-^{3}H]glucose and [U-^{14}C]alanine, steady-state plasma glucose and alanine turnover rates were calculated by dividing [^{3}H]glucose and [^{14}C]alanine infusion rates (dpm · kg^{-1} · min^{-1}) by their respective arterialized plasma glucose and alanine specific activities (dpm/μmol), as previously described (32). The percentage of plasma glucose derived from plasma alanine was calculated from the ratio of its specific activity corrected for differences in molecular weight. The rate at which plasma glucose was derived from plasma alanine was calculated as the product of the plasma turnover of glucose and the percentage of its derivation from alanine (32).

Statistical analysis. Data in text and figures are presented as means ± SE. The differences between the three groups were evaluated by analysis of variance corrected for repeated measures by means of a commercially available software package (CSS, Statsoft, Tulsa, OK). Correlations were assessed by least-squares linear regression (33).

RESULTS

Plasma glucose and insulin concentrations. Overnight (2:00–7:30 A.M.) and fasting (8:00–10:00 A.M.) plasma glucose

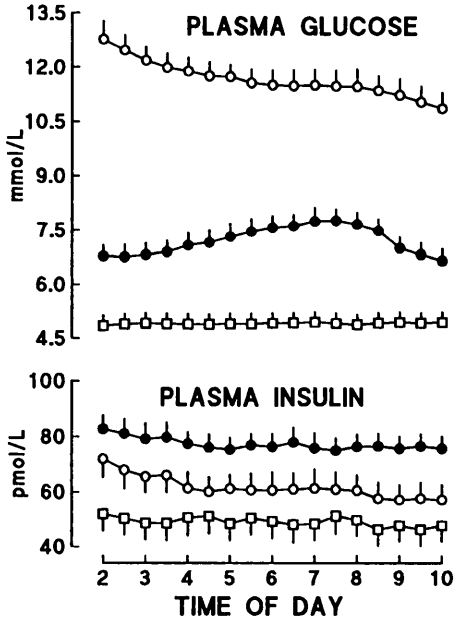

FIG. 1. Overnight plasma glucose and insulin concentrations in two groups of NIDDM patients and in control subjects. Data are means ± SE. Plasma glucose: $P < 0.05$ between NIDDM groups A and B and $P < 0.01$ between both NIDDM groups and control subjects. Plasma insulin: $P < 0.05$ between NIDDM group A and control subjects. □, normal subjects (n = 16); ●, NIDDM group A (n = 15); ○, NIDDM group B (n = 14).

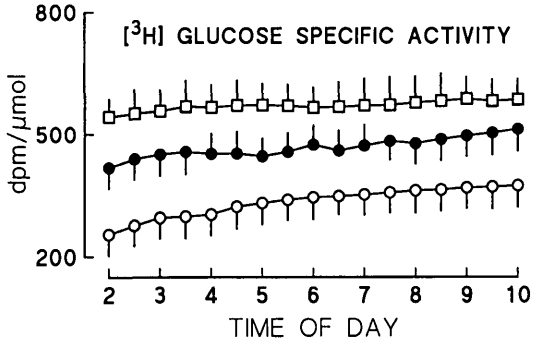

FIG. 2. Plasma [^{3}H]glucose specific activity in two groups of NIDDM patients and in control subjects. $P < 0.05$ between both NIDDM groups and control subjects. See Fig. 1 for definitions of symbols.

concentrations were different between the two groups of NIDDM subjects ($P < 0.05$) and were greater than in control subjects ($P < 0.01$; Fig. 1).

Plasma glucose concentrations increased progressively after 3:30 A.M. (6.9 ± 0.3 mmol/l) in patients of group A, reaching a peak of 7.8 ± 0.4 mmol/l at 7:30 A.M. ($P < 0.05$), and then decreased slightly to 6.6 ± 0.3 mmol/l at 10:00 A.M. In contrast, overnight plasma glucose concentrations decreased slightly in patients of group B from 11.9 ± 0.4 at 3:30 A.M. to 10.9 ± 0.4 at 10:00 A.M. and did not change in control subjects.

Despite these differences in plasma glucose concentrations, plasma insulin concentrations were significantly different only between the patients of group A and control subjects ($P < 0.05$), but there was no difference between those of group B and control individuals. Moreover, overnight plasma insulin concentrations did not change during the entire experimental period in both groups of NIDDM patients as well as in control subjects.

Plasma [3-^{3}H]glucose specific activity, systemic glucose production, and glucose utilization. Fasting (8:00–10:00 A.M.) plasma [^{3}H]glucose specific activity was lower in both groups of NIDDM patients (492 ± 52 and 365 ± 45 dpm/μmol for groups A and B, respectively) compared with control subjects (580 ± 61 dpm/μmol; $P < 0.05$; Fig. 2).

Plasma [^{3}H]glucose specific activity slightly increased in both groups of NIDDM patients until 3:30 A.M. and then remained stable until 10:00 A.M. There was no change throughout the study in control individuals.

Fasting (8:00–10:00 A.M.) SGP was lower in group A than in group B (11.1 ± 0.3 vs. 12.7 ± 0.3 μmol · kg^{-1} · min^{-1}, respectively; $P < 0.05$) but greater in both groups of diabetic subjects compared with control subjects (9.5 ± 0.3 μmol · kg^{-1} · min^{-1}; both $P < 0.05$; Fig. 3).

Overnight SGP initially decreased in both groups of NIDDM patients until 4:30 A.M. (12.2 ± 0.6 and 15.9 ± 1.2 μmol · kg^{-1} · min^{-1} at 2:00 A.M. and 11.3 ± 0.3 and 14.1 ± 0.5 μmol · kg^{-1} · min^{-1} at 4:30 A.M. for groups A and B, respectively; both $P < 0.05$). Afterward, SGP increased slightly to 11.6 ± 0.3 μmol · kg^{-1} · min^{-1} at 7:00 A.M. and then decreased to 10.1 ± 0.2 μmol · kg^{-1} · min^{-1} at 10:00 A.M. in group A (NS), whereas SGP decreased progressively in group B until 10:00 A.M. (12.2 ± 0.3 μmol · kg^{-1} · min^{-1}, $P < 0.05$). However, SGP remained greater in group B than in group A and nondiabetic subjects. In contrast, SGP did not change overnight in control individuals.

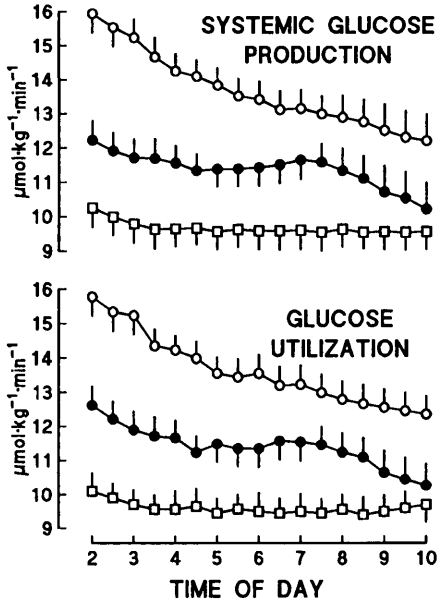

FIG. 3. Overnight systemic glucose production and utilization in two groups of NIDDM patients and in control subjects. Data are means ± SE. $P < 0.05$, NIDDM group A vs. NIDDM group B vs. control subjects. □, normal subjects ($n = 16$); ●, NIDDM group A ($n = 15$); ○, NIDDM group B ($n = 14$).

Overnight and fasting glucose utilization was greater in NIDDM patients compared with control subjects ($P < 0.05$) and followed the same pattern as SGP.

Fasting plasma glucose concentration, systemic glucose and alanine production, and GNG from alanine. In the 17 NIDDM patients ($n = 8$ and 9 from groups A and B,

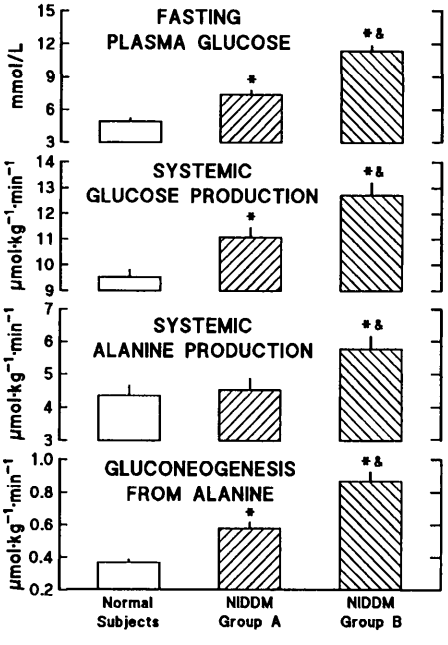

FIG. 4. Fasting plasma glucose concentration, systemic glucose and alanine production, and gluconeogenesis from alanine in two NIDDM groups and in control subjects. *$P < 0.05$ between both NIDDM groups and control subjects; &$P < 0.05$ between NIDDM groups A and B. □, $n = 10$; ▨, $n = 8$; ▧, $n = 9$.

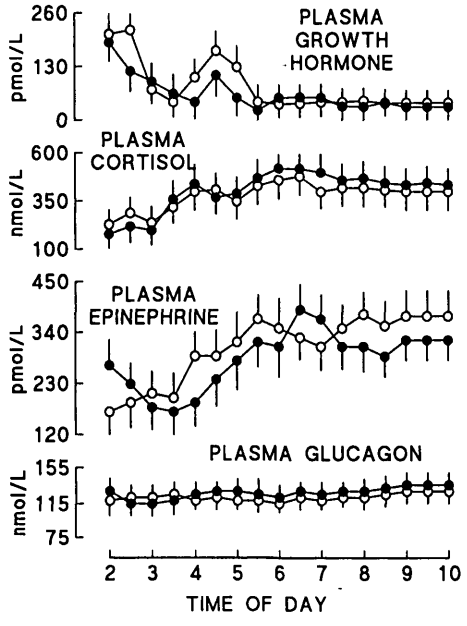

FIG. 5. Overnight plasma growth hormone, cortisol, epinephrine, and glucagon concentrations in NIDDM patients and control subjects. ●, NIDDM patients (n = 29); ○, normal subjects (n = 16).

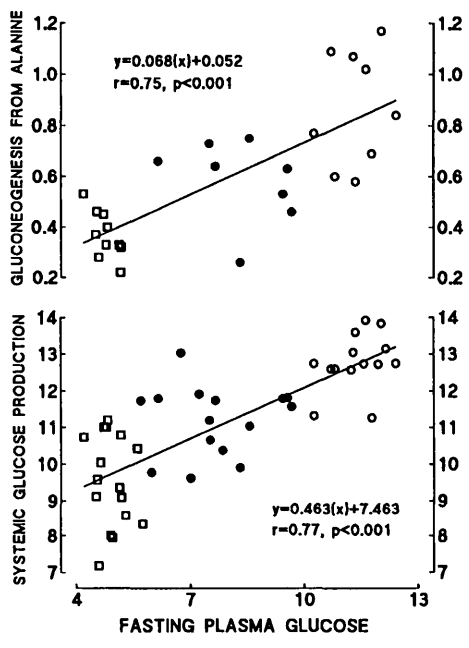

FIG. 6. Correlations between fasting (8:00–10:00 A.M.) plasma glucose and systemic glucose production and gluconeogenesis from alanine in the two groups of NIDDM patients and in control subjects. □, normal subjects; ●, NIDDM group A; ○, NIDDM group B.

respectively) and the 10 control subjects infused simultaneously with [3-^{3}H]glucose and [U-^{14}C]alanine, steady-state (8:00–10:00 A.M.) fasting plasma glucose concentrations, SGP, and GNG were different between the two groups of NIDDM patients (FPG 7.4 ± 0.2 and 11.4 ± 0.4 mmol/l, SGP 11.1 ± 0.4 and 12.7 ± 0.6 μmol · kg^{-1} · min^{-1}, and GNG 0.58 · 0.04 and 0.87 ± 0.06 μmol · kg^{-1} · min^{-1} for groups A and B, respectively; all $P < 0.05$; Fig. 4) and greater compared with control subjects (FPG 4.9 ± 0.2 mmol/l, SGP 9.5 ± 0.2 μmol · kg^{-1} · min^{-1}, GNG 0.37 ± 0.02 μmol · kg^{-1} · min^{-1}; all $P < 0.05$). However, systemic alanine production was greater in group B than in group A and control subjects (5.77 ± 0.41, 4.52 ± 0.35, and 4.36 ± 0.31 μmol · kg^{-1} · min^{-1}, respectively; both $P < 0.05$) but did not differ between group A and nondiabetic subjects (NS).

Plasma counterregulatory hormone concentrations. Overnight and fasting plasma glucagon, epinephrine, cortisol, and growth hormone concentrations did not differ in NIDDM patients (whole group) compared with control subjects (Fig. 5).

Correlations between FPG, SGP, and GNG from alanine. Fasting (8:00–10:00 A.M.) plasma glucose concentrations correlated with SGP (r = 0.77) and GNG from alanine (r = 0.75) in both groups of NIDDM patients as well as in control subjects (both $P < 0.01$; Fig. 6).

DISCUSSION

Inasmuch as SGP has not been found increased in an early stage of NIDDM, whereas glucose clearance (glucose utilization divided by plasma glucose concentration) is reduced (14–16), an inappropriately low (muscle) glucose uptake for prevailing plasma glucose and insulin concentrations has been considered primarily responsible for initiation of hyperglycemia (14). However, even with the above view assumed to be correct, an SGP "normal" in absolute terms in the presence of hyperglycemia and hyperinsulinemia would still suggest increased SGP, because normally hyperglycemia and hyperinsulinemia suppress SGP (34,35). In addition, according to the view of the primacy of decreased glucose uptake—not increased SGP—as the mechanism of fasting hyperglycemia, the pathogenesis of elevated FPG would differ at different stages of the natural history of NIDDM, because SGP and GNG have been found to be increased in severely hyperglycemic NIDDM patients (20,21).

We undertook the present series of studies to assess whether SGP is increased and/or glucose uptake is impaired in NIDDM patients without frankly overt fasting hyperglycemia (<7.8 mmol/l). In contrast to most (14–16) although not all (12) studies, we found that increased SGP is an early event in the natural history of NIDDM. In addition, we also found that GNG is increased in an early stage of NIDDM. Thus overproduction of glucose appears to be the primary determinant of fasting hyperglycemia in NIDDM, from the early stage of mild elevation in FPG throughout its progression to frankly overt hyperglycemia.

Several reasons may help explain different results obtained in previously published studies (14–16). In one study (14), the experimental data had been accumulated over several years, and the rates of glucose flux had to be readjusted in subjects studied before a given year. In addition, the cohort of NIDDM patients was not well matched for age, weight, or degree of obesity with control subjects. Of note, this conclusion has been based on the observation that "calculated" glucose clearance, not "true" and "absolute" rates of glucose utilization, is decreased in NIDDM patients with mild hyperglycemia (14). However, because glucose clearance is not independent of plasma glucose concentration, it decreases when prevailing plasma glucose increases (36). In fact, several studies have indicated that glucose clearance decreases nearly 30% if plasma glucose is increased in nondiabetic subjects to values similar to those of diabetic subjects

(37–39), most likely because brain glucose uptake, which is non–insulin mediated, does not increase in response to hyperglycemia (40–43). These considerations restrain the significance of the results of the above-mentioned study (14) and in general make it difficult to interpret results of studies based on glucose clearance.

In another study (15), SGP has not been found to be increased in NIDDM patients without fasting hyperglycemia. However, the relevance of such an observation remains uncertain because the same authors did not find excessive SGP in frankly hyperglycemic (>15 mmol/l) NIDDM patients (13), whereas they found an increase in SGP after insulin-induced reduction of plasma glucose to 7 mmol/l (44). More recently, it has been shown that eight NIDDM patients with a mean FPG of 6.2 mmol/l had rates of SGP similar to those of control individuals (16). However, in the same study (16), SGP and plasma glucose concentrations were closely correlated in NIDDM patients with FPG <6.6 mmol/l, indicating the primary role of "normal" SGP in determining FPG.

Moreover, in the above studies (14–16), SGP was determined in the morning, over approximately a 2-h period, assuming that the isotopic steady state had been reached only after 2 or 3 h of tracer equilibration. This assumption may be valid in control individuals, but it is not in NIDDM patients, who have an expanded glucose pool, and plasma glucose declines at the rate of ~0.52 mmol $\cdot l^{-1} \cdot h^{-1}$ during morning hours (18,45), thus making difficult the calculation of SGP and the comparison between diabetic and control individuals. In two of the three studies (15,16), the one-compartment, constant-pool-size, non-steady-state equation of Steele (45a) was used to overcome this problem. However, it is well established that when Steele's equations are employed, a lower estimate of SGP can result, thus masking the occurrence of increased SGP in NIDDM patients with FPG <7.8 mmol/l. Of note, the brief period of observation (30–45 min) gives only a static view of the dynamic changes in plasma glucose concentration and turnover that occur in NIDDM patients in postabsorptive conditions (45).

In the present series of studies, we employed a primed (proportional to plasma glucose concentrations) continuous infusion of [3-^{3}H]glucose for 12 h to rapidly reach and maintain an isotopic steady state throughout the observation period in control as well as diabetic individuals. In fact, from 3:30 A.M. until the end of study, the [^{3}H]glucose specific activity was constant in all subjects (Fig. 2). We believe that under these conditions the calculated SGP should closely reflect the true SGP. On the other hand, our approach may lead to prelabel glycogen with tritiated glucose during glucose cycling and consequent release of radioactive glucose along with cold glucose from the liver. This might falsely increase [^{3}H]glucose specific activity. However, if glucose cycling occurs, it can account for only 14% of SGP and should determine an underestimation of SGP, thus under- rather than overestimating the difference observed in the present study between diabetic and control individuals (46).

Recently, Jeng et al. (19) used an approach similar to the one of the present study but were not able to demonstrate any difference in SGP between NIDDM patients with FPG <10 mmol/l and control individuals. The exact reasons for their opposite result are not readily evident. However, several considerations may explain the controversial findings. First of all, in that study the prime of glucose tracer was not adjusted to prevailing plasma glucose, thus delaying the time of isotopic steady state in NIDDM patients to approximately 6:00 A.M. In contrast, in our study, in which the prime was adjusted, the steady state was reached at 3:30 A.M. (Fig. 2). Thus, in the study by Jeng and colleagues (19), SGP rates are difficult to interpret between 12:00 and 6:00 A.M. and probably mask the occurrence of the dawn increase in SGP (see below). Of note, in the same group these researchers studied NIDDM patients with FPG greater than in the present study (group A) and found normal SGP (19). However, in that study a role of SGP cannot be excluded because FPG was directly correlated to SGP in all groups of NIDDM subjects. This indirectly suggests a role of SGP in the development of FPG.

A new and interesting finding of the present study is the demonstration of an increased GNG from alanine in NIDDM patients with FPG <7.8 mmol/l. In general, GNG from alanine may be increased because of greater availability of alanine and/or greater efficiency of its conversion to glucose. In the present study, systemic appearance of alanine was increased only in NIDDM patients with overt fasting hyperglycemia (group B) and not in those with FPG <7.8 mmol/l (group A). Therefore it is likely that increased efficiency of GNG primarily contributes to exaggerate systemic glucose production in NIDDM patients without fasting hyperglycemia. This observation may have two relevant outcomes for the pathogenesis of NIDDM. First, increased GNG appears to be a significant metabolic alteration involved in the initiation of fasting hyperglycemia in NIDDM. Second, greater efficiency of GNG can be considered an early abnormality in NIDDM, because it is present in NIDDM patients with FPG <7.8 mmol/l. In NIDDM of longer duration and more severe fasting hyperglycemia, increased substrate (alanine) availability occurs. This is determined by hyperglycemia that increases by "mass effect" glucose uptake and its conversion to alanine in peripheral tissues other than muscle (47,48). Thus the present studies confirm that increased GNG remains the most important pathogenetic factor responsible for the development of fasting hyperglycemia in NIDDM. Regarding the mechanisms for increased efficiency of GNG, insulin resistance as well as increased free fatty acid oxidation could promote greater conversion of alanine to glucose (5,49).

In the present study, overnight plasma glucose concentration increased in NIDDM patients with fasting plasma glucose <7.8 mmol/l from 3:30 A.M., reaching a peak at 7:30 A.M., and then decreased slightly until 10:00 A.M. SGP followed a similar pattern. In contrast, overnight plasma glucose concentration and SGP progressively decreased from 3:30 to 10:00 A.M. in more hyperglycemic diabetic patients. In the two groups of NIDDM subjects, the different pattern of overnight glucose profile suggests that a "dawn" increase in plasma glucose concentration may be considered as a characteristic of less hyperglycemic NIDDM patients. Jeng et al. (19) found a similar pattern of plasma glucose, but they missed the early morning increase in SGP that we found, probably because they reached the isotopic steady state only at 6:00 A.M., whereas in our study [^{3}H]glucose specific activity was stable already at 3:30 A.M. The plasma glucose increase in the early morning hours has been called the dawn phenomenon, and it has been observed in IDDM patients as well as in control individuals (51–53). Although the dawn phenomenon has been described previously in NIDDM patients (51,54–57), the present study demonstrates that this event may be con-

sidered an early phenomenon in NIDDM that disappears when hyperglycemia further deteriorates. Moreover, this study shows that the dawn phenomenon is associated and possibly determined by a progressive increase in SGP from 3:30 to 7:00 A.M.

In the present study, the overnight increase in plasma glucose concentration in NIDDM patients without overt hyperglycemia was not accompanied by an increase in plasma insulin concentration. This lack of increase in plasma insulin concentration, which accounts for failure of SGP to decrease, indicates that an important impairment in insulin secretion is evident in an early phase of NIDDM (58,59). Overnight plasma insulin concentrations in this group of diabetic subjects were greater than those of nondiabetic subjects, but insulin secretion did not increase appropriately in response to an overnight increase in plasma glucose concentrations; this strongly suggests that fasting hyperinsulinemia is an indicator not only of insulin resistance (59,60) but also of failure of β-cells to respond promptly to increasing FPG.

In conclusion, increased SGP and GNG may be considered early determinants of fasting hyperglycemia in NIDDM. These abnormalities are secondary to insulin resistance in the liver and likely in the kidney (8). Lack of appropriate increase in insulin secretion in response to the increase in plasma glucose is an additional mechanism of fasting hyperglycemia in NIDDM. In contrast, reduced glucose clearance, most likely due to an inappropriate increase in brain glucose uptake, is the consequence, not the cause, of hyperglycemia due to exaggerated production of glucose. In addition, this study indicates that the mechanisms of fasting hyperglycemia do not change in the natural history of NIDDM but differ only quantitatively over time due to progressive deterioration of endogenous insulin secretion and increased free fatty acid availability.

ACKNOWLEDGMENTS

This work was supported by the Consiglio Nazionale delle Ricerche (Consiglio Nazionale delle Ricerche Grant 93.00352 and finalized project "Aging" grant).

The excellent technical help of Giampiero Cipiciani, Romeo Pippi, M. Chiara Aglietti, and Debora Mughetti is gratefully acknowledged.

REFERENCES

1. Koltermann OG, Gray RS, Griffin J, Brunstein P, Insel J: Receptor and postreceptor defects contribute to the insulin resistance in non-insulin-dependent diabetes mellitus. *J Clin Invest* 68:957–969, 1981
2. DeFronzo RA, Simonson D, Ferrannini E: Hepatic and peripheral insulin resistance: a common feature of type 2 (non-insulin-dependent) and type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 23:313–318, 1982
3. Reaven GM, Doberne L, Greenfield MS: Comparison of insulin secretion and in vivo insulin action in nonobese and moderately obese individuals with non-insulin-dependent diabetes mellitus. *Diabetes* 31:382–384, 1982
4. Bogardus C, Lillioja S, Howard B, Reaven G, Mott D: Relationship between insulin secretion, insulin action, and fasting plasma glucose concentration in nondiabetic and noninsulin-dependent diabetic subjects. *J Clin Invest* 74:1238–1246, 1984
5. Campbell PJ, Mandarino LJ, Gerich JE: Quantification of the relative impairment in actions of insulin on hepatic glucose production and peripheral glucose uptake in non-insulin-dependent diabetes mellitus. *Metabolism* 37:15–21, 1988
6. Chen Y-Di, Jeng C-Y, Hollenbeck CB, Wu M-S, Reaven GM: Relationship between plasma glucose and insulin concentration, glucose production, and glucose disposal in normal subjects and patients with non-insulin-dependent diabetes. *J Clin Invest* 82:21–25, 1988
7. Hother-Nielsen O, Beck-Nielsen H: On the determination of basal glucose production rate in patients with type 2 (non-insulin-dependent) diabetes mellitus using primed-continuous 3-^{3}H-glucose infusion. *Diabetologia* 33:603–610, 1990
8. Stumvoll M, Chintalapudi U, Perriello G, Welle S, Gutierrez O, Gerich J: Uptake and release of glucose by the human kidney: postabsorptive rates and responses to epinephrine. *J Clin Invest* 96:2528–2533, 1995
9. Dinnen S, Gerich J, Rizza R: Carbohydrate metabolism in non-insulin-dependent diabetes mellitus. *N Engl J Med* 327:707–713, 1992
10. Consoli A: Role of liver in the pathogenesis of NIDDM. *Diabetes Care* 15:368–381, 1992
11. De Fronzo RA, Bonadonna RC, Ferrannini E: Pathogenesis of NIDDM. A balanced overview. *Diabetes Care* 15:318–368, 1992
12. Gerich JE: Is muscle the major site of insulin resistance in type 2 (non-insulin-dependent) diabetes mellitus? *Diabetologia* 34:607–610, 1991
13. Beck-Nielsen H, Hother-Nielsen O, Vaag A, Alford F: Pathogenesis of type 2 (non-insulin-dependent) diabetes mellitus: the role of skeletal muscle glucose uptake and hepatic glucose production in the development of hyperglycemia. A critical comment. *Diabetologia* 37:217–221, 1994
14. De Fronzo R, Ferrannini E, Simonson D: Fasting hyperglycaemia in noninsulin-dependent diabetes mellitus: contributions of excessive hepatic glucose production and impaired tissue glucose uptake. *Metabolism* 38:387–395, 1989
15. Hother-Nielsen O, Beck-Nielsen H: Insulin resistance, but normal basal rates of hepatic glucose production in patients with newly diagnosed mild diabetes mellitus. *Acta Endocrinol* 124:637–645, 1991
16. Fery F: Role of hepatic glucose production and glucose uptake in the pathogenesis of fasting hyperglycemia in type 2 diabetes: normalization of glucose kinetics by short-term fasting. *J Clin Endocrinol Metab* 78:536–542, 1994
17. Reaven GM: The fourth musketeer—from Alexandre Dumas to Claude Bernard. *Diabetologia* 38:3–13, 1995
18. Chen Y-Di, Swislocki ALM, Jeng C-Y, Juang J-H, Reaven GM: Effect of time on measurement of hepatic glucose production. *J Clin Endocrinol Metab* 67:1084–1088, 1988
19. Jeng C-Y, Sheu WHH, Fuh MMT, Chen Y-D, Reaven GM: Relationship between hepatic glucose production and fasting plasma glucose concentration in patients with NIDDM. *Diabetes* 43:1440–1444, 1994
20. Consoli A, Nurjhan N, Capani F, Gerich J: Predominant role of gluconeogenesis in increased hepatic glucose production in NIDDM. *Diabetes* 38:550–557, 1989
21. Magnusson I, Rothman DL, Katz LD, Shulman RG, Shulman GI: Increased rate of gluconeogenesis in type II diabetes mellitus. *J Clin Invest* 90:1323–1327, 1992
22. World Health Organization: *Diet, Nutrition and the Prevention of Chronic Diseases: Report of a WHO Study Group*. Geneva, World Health Org., 1990, p. 69–74 (Tech. Rep. Ser. no. 797)
23. National Diabetes Data Group: Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. *Diabetes* 28:1039–1057, 1979
24. McGuire E, Helderman J, Tobin R, Andres R, Berman M: Effects of arterial versus venous sampling on analysis of glucose kinetics in man. *J Appl Physiol* 41:565–573, 1976
25. Lowry O, Passonneau J: Typical fluorometric procedures for metabolite assays. In *A Flexible System for Enzymatic Analysis*. Lowry O, Passonneau J, Eds. New York, Academic, p. 89–92
26. Fanelli C, Calderone S, Epifano L, De Vincenzo A, Modarelli F, Pampanelli S, Perriello G, De Feo P, Brunetti P, Gerich JE, Bolli GB: Demonstration of a critical role for free fatty acids in mediating counterregulatory stimulation of gluconeogenesis and suppression of glucose utilization in humans. *J Clin Invest* 92:1617–1622, 1993
27. Fanelli C, De Feo P, Porcellati F, Perriello G, Torlone E, Santeusanio F, Brunetti P, Bolli GB: Adrenergic mechanisms contribute to the late phase of hypoglycemic glucose counterregulation in humans by stimulating lipolysis. *J Clin Invest* 89:2005–2013, 1992
28. Perriello G, Misericordia P, Volpi E, Pampanelli S, Santeusanio F, Brunetti P, Bolli GB: Contribution of obesity to insulin resistance in NIDDM. *J Clin Endocrinol Metab* 80:2464–2469, 1995
29. Cole RA, Soeldner JS, Dunn PJ, Bunn HF: A rapid method for the determination of glycosylated hemoglobins using high pressure liquid chromatography. *Metabolism* 27:289–301, 1978
30. Perriello G, Jorde R, Nurjhan N, Stumvoll M, Dailey G, Jenssen T, Bier DM, Gerich JE: Estimation of the glucose-alanine-lactate-glutamine cycles in postabsorptive man: role of skeletal muscle. *Am J Physiol* 269:E443–E450, 1995

30a. De Bodo R, Steele R, Altzuler N, Dunn A, Bishop J: On the hormonal regu-

lation of carbohydrate metabolism: studies with C^{14} glucose. *Recent Prog Horm Res* 19:445–4489, 1963

30b. Miles J, Haymond M, Gerich J: Effects of free fatty acids, insulin, glucagon and adrenaline on ketone body production in humans. In *Metabolic Acidosis*. CIBA Foundation Symposium 87. London, Pitman Books, 1982, p. 192–213

31. Perriello G, Misericordia P, Volpi E, Santucci A, Santucci C, Ferrannini E, Ventura MM, Santeusanio F, Brunetti P, Bolli GB: Acute antihyperglycemic mechanisms of metformin in NIDDM. Evidence for suppression of lipid oxidation and hepatic glucose production. *Diabetes* 43:920–928, 1994
32. Nurjhan N, Bucci A, Perriello G, Stumvoll M, Dailey G, Bier DM, Toft I, Jenssen TG, Gerich JE: Glutamine: a major gluconeogenic precursor and vehicle for interorgan carbon transport in man. *J Clin Invest* 95:272–277, 1995
33. Zar J: *Biostatistical Analysis*. Englewood Cliffs, NJ, Prentice Hall, 1974
34. Sacca L, Hendler R, Sherwin RS: Hyperglycemia inhibits glucose production in man independent of changes in glucoregulatory hormones. *J Clin Endocrinol Metab* 47:1160–1167, 1978
35. Prager R, Wallace P, Olefsky JM: In vivo kinetics of insulin action on peripheral glucose disposal and hepatic glucose output in normal and obese subjects. *J Clin Invest* 78:472–481, 1986
36. Gerich JE: Control of glycaemia. *Baillieres Clin Endocrinol Metab* 7:551–587, 1993
37. Verdonk CA, Rizza RA, Gerich JE: Effects of plasma glucose concentration on glucose utilization and glucose clearance in normal man. *Diabetes* 30:535–537, 1981
38. Best J, Beard J, Taborsky G, Halter J, Porte D: Effect of hyperglycemia per se on glucose disposal and clearance in noninsulin-dependent diabetics. *J Clin Endocrinol Metab* 56:819–823, 1983
39. Yki-Jarvinen H, Young A, Lamkin C, Foley J: Kinetics of glucose disposal in whole body and across the forearm in man. *J Clin Invest* 79:1713–1719, 1987
40. Gerich J, Mitrakou A, Kelley D, Mandarino L, Nurjhan N, Reilly J, Jenssen T, Veneman T, Consoli A: Contribution of impaired muscle glucose clearance to reduced postabsorptive systemic glucose clearance in NIDDM. *Diabetes* 39:211–216, 1990
41. Reinmuth OM, Scheinberg P, Bourne B: Total cerebral flow and metabolism. *Arch Neurol* 12:49–66, 1965
42. Huang SC, Phelps ME, Hoffman EJ, Sideris K, Selin CJ, Kuhl DE: Non-invasive determination of local cerebral metabolic rate of glucose in man. *Am J Physiol* 238:E69–E82, 1980
43. Grill V: A comparison of brain glucose metabolism in diabetes as measured by positron emission tomography or by arteriovenous techniques. *Ann Med* 22:171–175, 1990
44. Vaag A, Alford F, Henriksen FL, Christopher M, Beck-Nielsen H: Multiple defects of both hepatic and peripheral intracellular glucose processing contribute to the hyperglycemia of NIDDM. *Diabetologia* 38:326–336, 1995
45. Glauber H, Wallace P, Brechtel G: Effects of fasting on plasma glucose and prolonged tracer measurement of hepatic glucose output in NIDDM. *Diabetes* 36:1187–1194, 1987

45a. Steele R, Wall JS, DeBodo RC, Altszuler N: Measurement of size and turnover rate of body glucose pool by the isotope dilution method. *Am J Physiol* 187:15–24, 1956

46. Efendic S, Karlander A, Vranic M: Mild type II diabetes markedly increases glucose cycling in the postabsorptive state and during glucose infusion irrespective of obesity. *J Clin Invest* 81:1953–1961, 1988
47. Consoli A, Nurjhan N, Reilly JJ, Bier DM, Gerich JE: Mechanism of increased gluconeogenesis in noninsulin-dependent diabetes mellitus. Role of alterations in systemic, hepatic, and muscle lactate and alanine metabolism. *J Clin Invest* 86:2038–2045, 1990
48. Stumvoll M, Perriello G, Nurjhan N, Bucci A, Welle S, Jansson P-A, Dailey G, Bier D, Jenssen T, Gerich J: Glutamine and alanine metabolism in NIDDM. *Diabetes* 45:863–868, 1996
49. Perriello G, Mitrakou A, Gumbiner B, Gerich JE: Non-esterified fatty acids in non-insulin-dependent diabetes: a critical update. *Diabetes Annu* 9:91–106, 1995
50. Bolli GB, Gerich JE: The dawn phenomenon: a common occurrence in both non-insulin-dependent and insulin-dependent diabetes mellitus. *N Engl J Med* 310:746–750, 1984
51. Bolli GB, De Feo P, De Cosmo S, Perriello G, Ventura MM, Calcinaro F, Lolli C, Campbell P, Brunetti P, Gerich JE: Demonstration of a dawn phenomenon in normal human volunteers. *Diabetes* 33:1150–1153, 1984
52. Perriello G, De Feo P, Torlone E, Panelli E, Santeusanio F, Brunetti P, Bolli GB: Nocturnal spikes of growth hormone secretion cause the dawn phenomenon in type 1 diabetes by decreasing hepatic (and extrahepatic) sensitivity to insulin in the absence of insulin waning. *Diabetologia* 33:52–59, 1990
53. Atiea JA, Ryder RRJ, Vora J, Owens DR, Luzio SD, Williams S, Hayes TM: Dawn phenomenon: its frequency in non-insulin-dependent diabetic patients on conventional therapy. *Diabetes Care* 10:461–465, 1987
54. Dimitriadis G, Vlachonikolis IG, Hatziagellaki E, Linos A, Kordonouri O, Alexopoulos E, Raptis S: The "dawn phenomenon" in patients with type II diabetes mellitus. 1:37–41, 1988
55. Atiea JA, Luzio S, Owens DR: The dawn phenomenon and diabetes control in treated NIDDM and IDDM patients. *Diabetes Res Clin Pract* 16:183–190, 1992
56. Jasik M, Orlowska K: Occurrence of early-morning hyperglycemia (dawn phenomenon) in patients with diabetes mellitus type 1 and 2. *Pol Arch Med Wewn* 81:207–213, 1989
57. Leahy JL: Natural history of beta cell dysfunction in NIDDM. *Diabetes Care* 13:992–1010, 1990
58. Porte D: Beta cells in type II diabetes mellitus. *Diabetes* 40:166–180, 1991
59. Saad MF, Knowler WC, Pettitt DJ, Nelson RG, Mott DM, Bennett PH: Sequential changes in serum insulin concentration during development of non-insulin-dependent diabetes. *Lancet* i:1356–1359, 1989
60. Lillioja S, Nyomba BL, Saad MF, et al.: Exaggerated early insulin release and insulin resistance in a diabetes-prone population: a metabolic comparison of Pima Indians and Caucasians. *J Clin Endocrinol Metab* 73:866–876, 1991