Postprandial Hyperglycemia in Patients with Noninsulin-dependent Diabetes Mellitus

Role of Hepatic and Extrahepatic Tissues

R. G. Firth, P. M. Bell, H. M. Marsh,* I. Hansen, R. A. Rizza
Endocrine Research Unit, Departments of Internal Medicine and Anesthesiology, Mayo Clinic, Rochester, Minnesota 55905*

Abstract

Patients with noninsulin-dependent diabetes mellitus (NIDDM) have both preprandial and postprandial hyperglycemia. To determine the mechanism responsible for the postprandial hyperglycemia, insulin secretion, insulin action, and the pattern of carbohydrate metabolism after glucose ingestion were assessed in patients with NIDDM and in matched nondiabetic subjects using the dual isotope and forearm catheterization techniques. Prior to meal ingestion, hepatic glucose release was increased ($P < 0.001$) in the diabetic patients measured using [2-^{3}H] or [3-^{3}H] glucose. After meal ingestion, patients with NIDDM had excessive rates of systemic glucose entry (1,316±56 vs. 1,018±65 mg/kg · 7 h, $P < 0.01$), primarily owing to a failure to suppress adequately endogenous glucose release (680±50 vs. 470±32 mg/kg · 7 h, $P < 0.01$) from its high preprandial level. Despite impaired suppression of endogenous glucose production during a hyperinsulinemic glucose clamp ($P < 0.001$) and decreased postprandial C-peptide response ($P < 0.05$) in NIDDM, percent suppression of hepatic glucose release after oral glucose was comparable in the diabetic and nondiabetic subjects (45±3 vs. 39±2%). Although new glucose formation from meal-derived three-carbon precursors (53±3 vs. 40±7 mg/kg · 7 h, $P < 0.05$) was greater in the diabetic patients, it accounted for only a minor part of this excessive postprandial hepatic glucose release. Postprandial hyperglycemia was exacerbated by the lack of an appropriate increase in glucose uptake whether measured isotopically or by forearm glucose uptake. Thus as has been proposed for fasting hyperglycemia, excessive hepatic glucose release and impaired glucose uptake are involved in the pathogenesis of postprandial hyperglycemia in patients with NIDDM.

Introduction

After ingestion of glucose both hepatic and extrahepatic tissues participate in the maintenance of normal carbohydrate tolerance. In nondiabetic humans, the liver minimizes postprandial hyperglycemia both by increasing glucose uptake and by suppressing endogenous glucose production (1–3). Although 90–100% of the ingested glucose initially reaches the peripheral circulation (2, 3), ~30–35% is eventually cleared by the liver (4, 5). Recent studies have emphasized that hepatic glycogen formation may occur both by direct uptake of glucose or by synthesis of new glucose from three-carbon precursors (6–8). Postprandial suppression of endogenous glucose production can prevent an additional 25–35 g of glucose from entering the systemic circulation (2, 3). These processes presumably are orchestrated by both insulin- and glucose-induced modulation of glucose production and utilization.

Patients with noninsulin-dependent diabetes mellitus (NIDDM)[1] are hyperglycemic both prior to and after meal ingestion. A variety of studies have suggested that after an overnight fast, hyperglycemia is due to overproduction of glucose (9–12). These abnormalities presumably result from both insulin resistance (13–16) as well as a failure of compensatory pancreatic insulin secretion (17). In contrast to preprandial hyperglycemia, the mechanisms responsible for postprandial hyperglycemia are unknown. Felig et al. (18), using the hepatic catheter technique, suggested that postprandial glucose intolerance in patients with NIDDM was due to impaired hepatic uptake of ingested glucose. This conclusion, however, was based on the assumption that endogenous glucose production remained constant in both diabetic and nondiabetic humans. Although this assumption has subsequently been shown to be erroneous in nondiabetic man (2, 3), there exist no data examining the relative contributions of meal-related and endogenous glucose release to postprandial carbohydrate intolerance. Previous studies in patients with insulin-dependent diabetes mellitus demonstrated that the primary cause of postprandial hyperglycemia was the failure of the liver to decrease endogenous glucose release appropriately (3). Whether a similar defect is present in patients with NIDDM who have residual insulin secretion and therefore can increase portal venous insulin concentrations is unknown. Although it is well established that gluconeogenesis is increased in NIDDM patients in the postabsorptive state (19–21), there are no data determining whether the rate of new glucose formation from meal-derived three-carbon precursors is increased after meal ingestion. Finally, in addition to uncertainty regarding the pattern of postprandial hepatic glucose release, the contribution of alterations in glucose uptake by extrahepatic tissues to impaired postprandial meal disposition in NIDDM also is unknown.

The current studies were, therefore, undertaken to address each of these issues. We sought to determine (*a*) whether postprandial hyperglycemia is due to excessive glucose entry or decreased glucose utilization, (*b*) if excessive postprandial glucose entry occurs, whether this is due to a failure to suppress endogenous glucose production adequately and/or to increased rates of systemic meal glucose appearance, (*c*) whether systemic entry of glucose formed from meal-derived three-carbon precursors is increased, (*d*) whether alterations in total body glucose utilization are accompanied by alterations in extrahepatic insulin-sensitive tissues as exemplified by the forearm, and (*e*) whether the ab-

Address reprint requests to Dr. Rizza.

Received for publication 16 July 1985 and in revised form 15 January 1986.

J. Clin. Invest.

0021-9738/86/05/1525/08 $1.00
Volume 77, May 1986, 1525–1532

1. *Abbreviations used in this paper:* CV, coefficient of variation; GH, growth hormone; NIDDM, noninsulin-dependent diabetes mellitus.

normal pattern of postprandial glucose metabolism is associated with abnormalities in insulin secretion and/or action.

Methods

All studies were performed in the Clinical Research Center using protocols approved by the Mayo Clinic Institutional Review Board. Informed, written consent was obtained from 13 noninsulin-dependent diabetic patients and seven nondiabetic subjects (Table I). All subjects were otherwise healthy. Of the 13 diabetic patients, nine were being managed with diet alone, and four were receiving sulfonylureas in addition. Sulfonylureas were discontinued at least 3 wk prior to study. All volunteers consumed a stable diet including at least 200 g of carbohydrate for the 3 d preceding study. Meal studies were performed first on all individuals followed 1 wk later by the hyperinsulinemic glucose clamp studies, except for one diabetic subject in whom this order was reversed. All studies were commenced after an overnight fast, the patients having consumed only water for the previous 12–14 h.

Meal study. On the morning of study, a large antecubital vein was cannulated with an 18-gauge plastic cannula (Cathlon IV, Criticon, Inc., Tampa, FL) for administration of [2-^{3}H]glucose tracer. A 19-gauge butterfly needle (Terumo Corp., Tokyo, Japan) was inserted in a dorsal vein of the ipsilateral hand which was maintained at 55°C for intermittent sampling of arterialized blood as previously described (22, 23). A second 18-gauge cannula was inserted in retrograde fashion into a large, deep antecubital vein of the contralateral arm for intermittent sampling of deep venous forearm blood (24). An electrocapacitance plethysomography cuff (UFI, Morro Bay, CA) was applied just distal to the tip of this cannula for measurement of forearm blood flow by the method of Figar (25) as modified by Hyman et al. (26). The venous occlusion cuff was fitted to the upper arm and a high pressure wrist cuff inflated to 300 mmHg to isolate the hand vasculature 3 min prior to blood flow measurement and deep venous sampling.

A primed (13.8 μCi) continuous (0.138 μCi/min) infusion of [2-^{3}H]glucose (New England Nuclear, Boston, MA; sp act 14 Ci/mmol) was commenced between 0700 and 0800 hours for isotopic determination of total rates of glucose appearance and uptake. After a 2-h equilibration period, each subject ingested 50 g of glucose labeled with 100 μCi [6-^{14}C]glucose (New England Nuclear; sp act 9.5 Ci/mmol) dissolved in 100 ml of artificially flavored water. Two subsequent water rinses of the flask were also ingested, after which <0.01% of the original radioactivity remained in the flask.

Forearm blood flow measurement and simultaneous blood sampling from the arterialized and deep venous sites were performed 30, 20, 10, and 0 min before and 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, and 420 min after glucose ingestion. Blood samples were immediately placed on ice, centrifuged at 4°C, and separated for storage at −20°C until assayed.

Blood for the determination of glucose specific activities and ^{14}C randomization was deproteinized by the method of Somogyi (27) and purified by sequential passage over anion (AGl-X8, Bio-Rad Laboratories, Richmond, CA) and cation (AG5OW, Bio-Rad Laboratories) exchange columns to remove charged intermediary metabolites. The eluate was then evaporated to dryness under nitrogen and reconstituted in phosphate buffer, pH 7.4. Plasma insulin, C peptide, and glucagon were determined by radioimmunoassay as previously described (28–30). Patients voided prior to and after completion of the study. Glycosuria over the 7 h was minimal in both diabetic (16.8±8.4 mg/kg · 7 h) and nondiabetic (0.42±0.42 mg/kg · 7 h) subjects.

After the ingestion of [6-^{14}C]glucose, radioactive glucose derived from the meal may appear in the peripheral blood as unchanged [6-^{14}C]glucose or as new glucose ultimately derived from the meal which previously had undergone glycolysis to and resynthesis from three-carbon intermediates. In such new glucose, redistribution of ^{14}C among carbon atoms 1, 2, 5, and 6 will be approximately equal (31, 32). Therefore, the amount of ^{14}C present in the first carbon of glucose was determined in each sample by a modification of the enzymatic decarboxylation method of Kalhan et al. (33). The assay was performed in 16-mm glass tubes (American Scientific Products, McGaw Park, IL) closed with tightly fitting rubber stoppers (Bittner Corp., Norcross, CA) in a total reaction volume of 2.2 ml containing 133 mM phosphate buffer, 0.31% bovine albumin, 6 mM magnesium chloride, 6.8 mM adenosine-5'-triphosphate, 6.8 mM nicotinamide adenine dinucleotide phosphate, 6 U of hexokinase [E.C. 2.7.1.1], 6 U of glucose-6-phosphate dehydrogenase (type XIII), 6.8 mM ammonium chloride, 9 mM alpha-ketoglurate, 6 U of 6-phosphogluconate dehydrogenase [E.C. 1.1.1.44] (all from Sigma Chemical Co., St. Louis, MO) and 15 U of glutamate dehydrogenase (Calbiochem-Behring Corp., LaJolla, CA). The reaction was initiated by injection of a mixture of the latter four reagents through the rubber stopper using a 3½-inch 20-gauge needle (Monoject, Sherwood Medical Industries, Inc., St. Louis, MO). Incubation time was 24 h at 37°C. This method gives quantitative decarboxylation of glucose (96.3±0.4%) in plasma samples containing glucose at concentrations ranging from 40 to 500 mg/dl. All assays were performed in duplicate. Intraassay coefficient of variation (CV) for decarboxylation was 2.7±1.0% and interassay CV 1.6%. Carbon dioxide derived from carbon atom 1 of the glucose was trapped in 1 M hydroxide of hyamine (J. T. Baker Chemical Co., Phillipsburg, NJ) contained in a plastic well (Kontes Glassware, Vineland, NJ) suspended from the rubber stopper. $^{14}CO_2$ thus trapped was determined by liquid scintillation counting. Trapping efficiency was 61.9±0.7% with an intraassay CV of 5.7±0.7% and interassay CV of 3.6%. The ingested [6-^{14}C]glucose was shown by this method to be contaminated with <0.32% [1-^{14}C]glucose (mean±SEM = 0.22±0.02%).

Total rates of glucose appearance and utilization were calculated using the equations of Steel et al. (34) as modified by DeBodo et al. (35). The rate of appearance in the circulation of any ^{14}C-labeled glucose (i.e., any glucose ultimately derived from the meal) ($^{14}CR_a$) was traced using [2-^{3}H]glucose as suggested by Cherrington et al. (36) and as previously described (3). Of this, new glucose appearance from meal-derived three-carbon compounds (RR_a) was assumed to be four times the rate of appearance of [1-^{14}C]glucose determined as by enzymatic decarboxylation (31, 32). Subtraction of RR_a from $^{14}CR_a$ yields the rate of appearance of noncatabolized [6-^{14}C]glucose derived directly from the meal (meal-derived glucose).

Forearm glucose uptake was obtained from the product of the forearm blood flow and the arteriovenous difference in whole blood glucose concentration. The latter is derived as follows: whole blood glucose concentration = plasma glucose concentration × (1–0.3 hematocrit) (37).

Modified meal study. To determine the extent to which futile cycling

Table I. Patient Characteristics

	Age	Sex	Weight	Body mass index	Fasting plasma glucose	Glyco Hb
	yr		*kg*	*kg/m²*	*mg/dl*	*%*
NIDDM (n = 13)	56±3	9 F, 4 M	84±4	31.3±1.7	187±11	9.8±0.6
Nondiabetic (n = 7)	51±4	6 F, 1 M	89±8	32.1±2.0	98±2	4.8±0.2
P value	NS	NS	NS	NS	<0.001	<0.001

All values mean±SEM. F, female; M, male.

at the level of glucose/glucose-6-phosphate and/or cycling of glucose through glycogen influenced the results in the diabetic patients, three patients were restudied using an experimental design identical to the original meal study with the exception that a primed-continuous infusion of both [2-^{3}H]glucose and [3-^{3}H]glucose was commenced between 0700 and 0800 hours. Total glucose appearance, systemic appearance of meal-derived glucose, endogenous glucose release, and new glucose appearance from meal derived three-carbon precursors were traced separately using each isotope. Forearm glucose uptake was not measured in these subjects.

Blood for determination of [2-^{3}H]- and [3-^{3}H]glucose specific activity was deproteinized and passed over anion and cation exchange columns as described above. After drying, one aliquot was used for determination of total ^{14}C and total ^{3}H counts. After addition of 0.5 ml of 133 mM phosphate buffer and 10 ml of Safety-Solve (Research Products International Corp., Mount Prospect, IL), this 0.25-ml aliquot was counted in a dual-channel refrigerated liquid scintillation spectrometer. The external ratios method was used to correct for quenching. Radioactivity in this aliquot represented total ^{14}C and total ^{3}H (both 2-^{3}H and 3-^{3}H radioactivity).

The second 0.25-ml aliquot was used for determination of [3-^{3}H]glucose radioactivity. This was accomplished by selective enzymatic detritiation of [2-^{3}H]glucose during which [3-^{3}H]glucose was left intact (38). Subtraction of the [3-^{3}H]glucose radioactivity from total tritiated radioactivity yielded [2-^{3}H]glucose radioactivity. Selective detritiation of [2-^{3}H]glucose was performed using a modification of the method of Issekutz (38). O.5 ml of a mixture containing 1.2 U of hexokinase (Sigma Chemical Co.), 5 U of phosphoglucose isomerase (Sigma Chemical Co.), $MgCl_2$ (final concentration in reaction mixture 6 mM), and ATP (Sigma Chemical Co., final concentration 5.6 mM) was added to a 0.25-ml aliquot of reconstituted plasma. The mixture was incubated at 37°C for 2 h. The samples were then dried under a stream of air, reconstituted in water, and counted as above.

External standards of [3-^{3}H]glucose and [2-^{3}H]glucose in patient plasma were processed in parallel with every patient assay. The results were used to calculate the degree of detritiation of each isotope during each patient assay. Overall completion of detritiation of [2-^{3}H]glucose was 96.5±0.3, (interassay CV 1.63%, intra-assay CV 0.9%) while 98.7±0.6 (interassay CV 2.6%, intra-assay CV 2.3%) of [3-^{3}H]glucose remained intact.

Hyperinsulinemic glucose clamp study. In order to quantitate hepatic and extrahepatic insulin action at insulin concentrations commonly seen postprandially, hyperinsulinemic clamp procedures were performed on all the nondiabetic volunteers and 12 of the 13 diabetic patients in the meal study. A primed- (16.7 μCi) continuous (0.167 μCi/min) infusion of [3-^{3}H]glucose (New England Nuclear; sp act 13.5 Ci/mmol) was commenced between 0700 and 0800 hours through an 18 gauge antecubital intravenous cannula (Cathlon IV). After a 2-h equilibration period, a primed- (4 mU/kg) continuous (0.4 mU/kg · min) infusion of semisynthetic regular human insulin (Actrapid, Squibb Novo Industries, Princeton, NJ) in 0.9% saline containing 1% human serum albumin (Cutter Laboratories, Berkeley, CA) was administered for a further 3 h in both diabetic and nondiabetic subjects. Plasma glucose was allowed to decrease in the diabetic patients until it reached euglycemic levels where it was maintained in both groups by an exogenous infusion of 50% dextrose as previously described (23).

Arterialized blood was obtained through a butterfly needle (Terumo Corp.) lying retrograde in a dorsal vein of the contralateral hand which was maintained at 55°C. Samples for glucose specific activity were obtained during the final 30 min prior to and during the insulin infusion (23) and at half-hourly periods throughout the study for determination of insulin (28), C peptide (29), glucagon (30), and growth hormone (GH) (39) concentrations.

Rates of total glucose appearance and utilization were calculated during the final 30 min of the clamp using the equations of Steele et al. (34) as modified by DeBodo et al. (35). Endogenous glucose production was calculated by subtracting the amount of glucose infused from the isotopically determined glucose production rate (23).

Data in the text and figures are given as mean±standard error of the mean. The statistical analyses were performed using Student's *t* tests for normally distributed data and the Mann-Whitney two-sample rank test for nonnormally distributed data (40). Correlation coefficients were calculated using linear regression analysis. A *P* value of <0.05 was considered to be statistically significant.

Results

Meal study

Plasma glucose, insulin, C peptide, and glucagon concentrations (Fig. 1). Prior to carbohydrate ingestion, the plasma glucose concentration was greater in the diabetic patients than in the nondiabetic subjects (187±11 vs. 98±3 mg/dl; $P < 0.001$). After carbohydrate ingestion both the peak plasma glucose concentration (280±12 vs. 149±5 mg/dl; $P < 0.001$) and glycemic excursion (95±8 vs. 51±5 mg/dl; $P < 0.001$) were greater and remained above preprandial levels longer (313±19 vs. 219±28 min; $P < 0.02$) in the diabetic patients than in the nondiabetic subjects. This resulted in a greater integrated glucose response above baseline in the diabetic patients (14,850±2,067 vs. 6,118±1,174 min · mg/dl; $P < 0.002$).

Plasma insulin concentration did not differ significantly either prior to (14±2 vs. 11±5 μU/ml) or during the 7 h after meal ingestion in the diabetic and nondiabetic subjects, respectively. Neither plasma C peptide concentration (2.1±0.2 vs. 2.2±0.6 ng/ml) prior to nor integrated responses during the 7 h after meal ingestion (1,192±137 vs. 1,495±280 ng/ml · 7 h) differed significantly in the diabetic and nondiabetic subjects. However, the integrated C-peptide response above baseline was less in the former (340±74 vs. 613±65 min · ng/ml; $P < 0.05$). Plasma glucagon concentration was similar in both groups throughout the study.

Total glucose appearance and uptake (Fig. 2). In the postabsorptive state, glucose appearance rates were greater in the diabetic patients than in nondiabetic subjects whether deter-

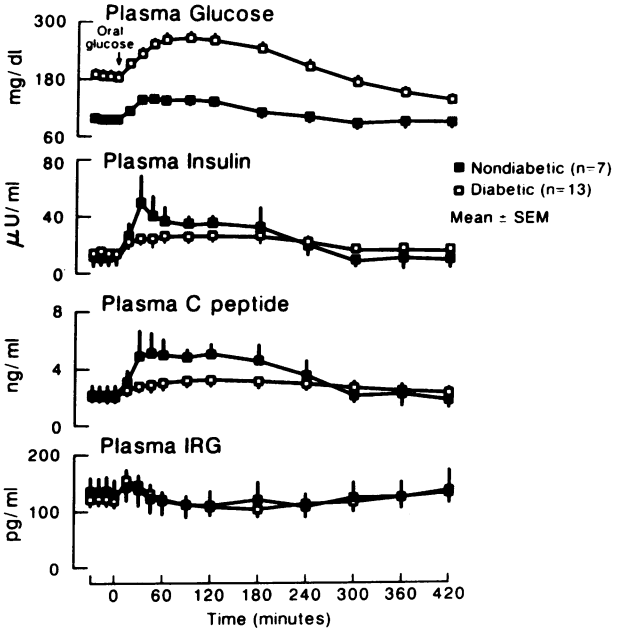

Figure 1. Plasma glucose, insulin, C peptide, and glucagon (IRG) concentrations in the postabsorptive state and after glucose ingestion (*arrow*).

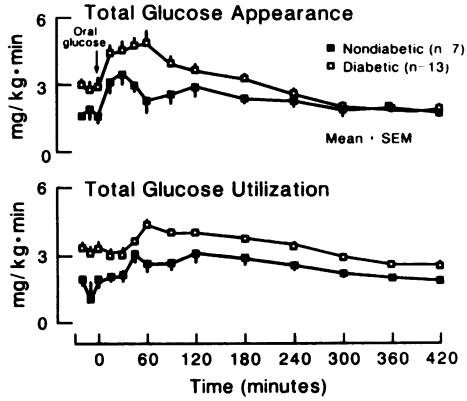

Figure 2. Total rates of systemic glucose appearance and utilization in the postabsorptive state and after glucose ingestion (*arrow*).

mined with [2-^{3}H]glucose (3.04±0.24 vs. 1.84±0.10 mg/kg·min; $P < 0.001$) or [3-^{3}H]glucose (2.34±0.13 vs. 1.43±0.08 mg/kg·min, $P < 0.001$). Glucose appearance rates in the diabetic patients were significantly correlated with fasting plasma glucose whether determined with [2-^{3}H] ($r = 0.67$, $P < 0.02$) glucose or [3-^{3}H]glucose ($r = 0.76$, $P < 0.01$). The integrated response over the 7 h after meal ingestion (1,316±56 vs. 1,018±65 mg/kg·7 h, $P < 0.01$) was greater in the diabetic patients.

Rates of glucose uptake prior to (3.21±0.23 vs. 1.92±0.09 mg/kg·min; $P < 0.001$) and during the 7 h after meal ingestion (1,351±56 vs. 1,023±64 mg/kg·7 h, $P < 0.01$) were also greater in the diabetic patients. However, the integrated response above basal was significantly less in the diabetic patients (122±32 vs. 236±45 mg/kg, $P < 0.05$).

Meal-derived, endogenous, and new glucose appearance from meal-derived three-carbon precursors (Fig. 3). The amount of meal-derived glucose reaching the systemic circulation over the 7-h observation period was slightly greater (622±19 vs. 521±15 mg/kg; $P < 0.02$) in the diabetic patients, primarily owing to a greater rate of entry during the first 2 h (259±22 vs. 194±27 mg/kg, $P = 0.08$).

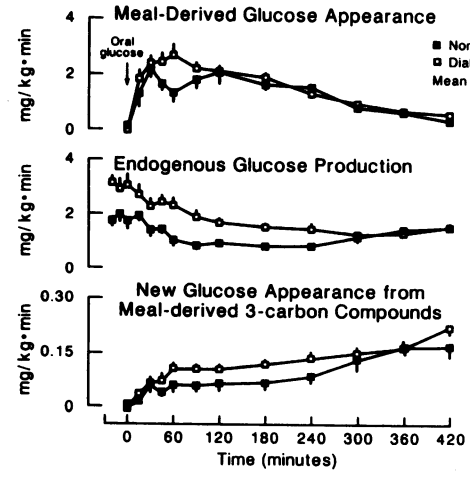

Figure 3. Rates of systemic appearance of meal-derived glucose, endogenous glucose production, and new glucose synthesized from meal-derived three-carbon intermediates (*arrow* indicates glucose ingestion).

After meal ingestion, endogenous glucose production decreased in the nondiabetic subjects to a nadir of 0.9±0.1 mg/kg·min at 90 min, increasing to 1.5±0.1 mg/kg·min at 420 min ($P < 0.005$ vs. 90 min). In contrast, the nadir in endogenous glucose production in diabetic patients did not occur until 300 min (1.3±0.1 mg/kg·min) with glucose production increasing only minimally thereafter to 1.6±0.1 mg/kg·min at 420 min ($P < 0.06$ vs. 180 min). Percent suppression below basal was similar in both groups (45±3 vs. 39±2%). However, owing to the higher basal rates, total endogenous glucose release over the 7 h after meal ingestion was greater in the diabetic patients (680±50 vs. 470±32 mg/kg·7 h, $P < 0.01$).

New glucose appearance from meal-derived three-carbon precursors increased progressively throughout the 7-h period in both the diabetic and nondiabetic subjects, reaching a maximum of 0.22±0.21 and 0.17±0.03 mg/kg·min, respectively. The total amount of glucose formed from meal-derived three-carbon precursors entering the systemic circulation over the 7-h postprandial period was slightly greater in the diabetic than nondiabetic subjects (53±3 vs. 40±7 mg/kg·7 h, $P < 0.05$) but did not differ when expressed as percentage of endogenous glucose release over the same interval (8.3±0.7 vs. 8.5±1.5%, respectively).

Because endogenous glucose release tended to rise in the nondiabetic patients from 4 h onward, presumably reflecting postprandial counterregulation (41), differences between diabetic and nondiabetic subjects may have been underestimated by analyzing the data over the full 7-h period of observation. The data, therefore, were also integrated over the initial 4 h. Over this interval, endogenous glucose release (434±35 vs. 241±16 mg/kg·4 h, $P < 0.0001$) and new glucose appearance from meal-derived three-carbon precursors (24±1 vs. 14±3 mg/kg·4 h, $P < 0.001$) remained greater in the diabetic than nondiabetic subjects. The amount of meal-related glucose reaching the systemic circulation did not differ significantly (477±31 vs. 401±45 mg/kg·4 h).

Comparison of meal disposal measured using [2-^{3}H]- and [3-^{3}H]glucose (Fig. 4)

To determine the extent to which the rsults in the diabetic patients were influenced by the use of [2-^{3}H]glucose to trace systemic glucose appearance, three diabetic patients were restudied. Experimental conditions were identical to those of the initial meal study with the exception that systemic glucose appearance was traced using a simultaneous infusion of both [2-^{3}H]- and [3-^{3}H]glucose. Preprandial plasma glucose (188±17 vs. 196±18 mg/dl) and insulin concentrations (19±6 vs. 20±6 μU/ml) were similar on the initial and repeat studies. When the simultaneous infusion of [2-^{3}H] and [3-^{3}H] was employed, postabsorptive glucose production rates were higher when determined with [2-^{3}H]glucose than when determined with [3-^{3}H]glucose (3.0±0.6 vs. 2.1±0.6 mg/kg·min). These observations are in agreement with those present when [2-^{3}H]- and [3-^{3}H]glucose were used to measure postabsorptive production rates on separate days as part of the original study. After glucose ingestion, changes in rates of total, meal-related, and endogenous glucose appearance as well as new glucose appearance from meal-derived precursors were comparable whether measured with [2-^{3}H]- or [3-^{3}H]glucose. Although not significantly different, postprandial endogenous glucose release measured with [3-^{3}H]glucose was slightly higher than those measured with [2-^{3}H]glucose in each individual.

Forearm glucose uptake (Fig. 5). Forearm blood flow did

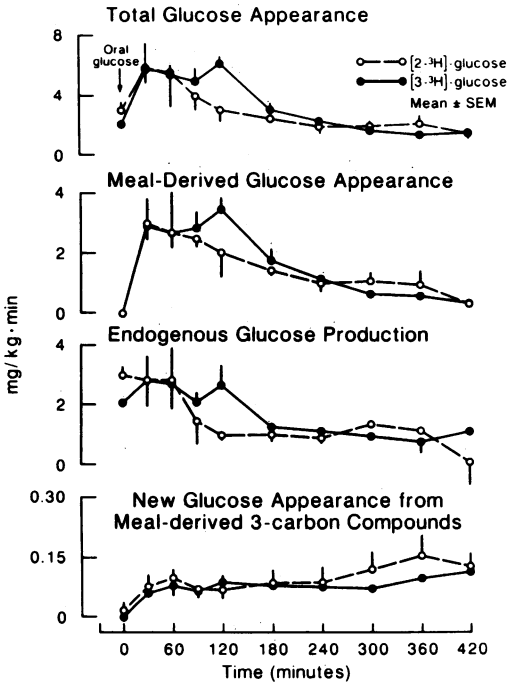

Figure 4. Rates of total glucose appearance, meal-derived glucose appearance, endogenous glucose production, and new glucose appearance from meal-derived three-carbon compounds, assessed using a simultaneous infusion of [2-^{3}H]- and [3-^{3}H]glucose.

not differ significantly in the diabetic and nondiabetic subjects either prior to (4.4±0.4 vs. 4.1±0.7 ml/dl forearm · min) or after (4.1±0.3 vs. 4.6±0.7 ml/dl forearm · min) meal ingestion. Forearm glucose uptake prior to meal ingestion was greater in the diabetic than nondiabetic subjects (0.26±0.04 vs. 0.14±0.02 mg/dl forearm · min, $P < 0.02$). Over the 7 h after meal ingestion, forearm glucose uptake was slightly but not significantly higher in the diabetic patients (127±22 vs. 106±11 mg/dl forearm · 7 h). However, the integrated increase in forearm glucose uptake above basal tended to be less in the diabetic than nondiabetic subjects (48±12 vs. 55±8 mg/dl forearm).

Effects of insulin on glucose production and glucose utilization (hyperinsulinemic clamp) (Fig. 6). Because glucose and insulin concentrations varied after meal ingestion in both diabetic and nondiabetic subjects, an accurate assessment of insulin action is difficult. Therefore the ability of insulin to suppress glucose production and stimulate glucose utilization was assessed in the same individuals on a separate day using the hyperinsulinemic glucose clamp technique. Plasma glucose concentration averaged

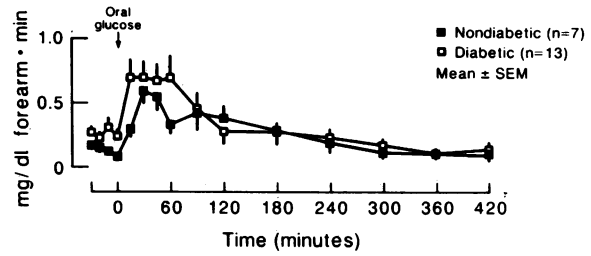

Figure 5. Rates of forearm glucose uptake in the postabsorptive state and after glucose meal (*arrow*).

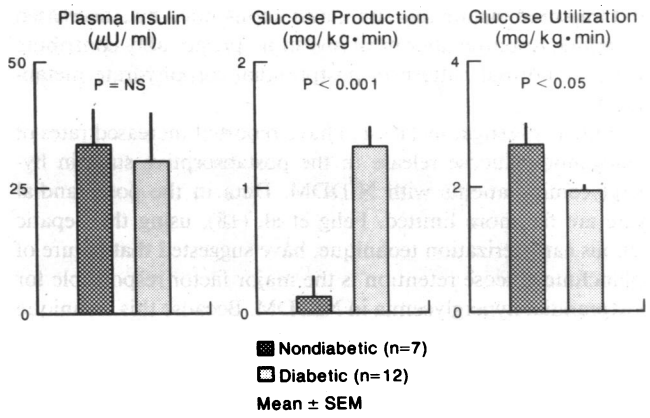

Figure 6. Plasma insulin concentration (*left*), rates of endogenous glucose production (*middle*), and rates of insulin-stimulated glucose utilization (*right*) during the final 30 min of the hyperinsulinemic glucose clamp study. Insulin was infused at the rate of 0.4 mU/kg · min.

193±11 and 99±2 mg/dl ($P < 0.001$) prior to infusion of insulin and 116±8 mg/dl and 92±1 mg/dl ($P < 0.01$) during the final 30 min of the insulin infusion in the diabetic and nondiabetic subjects, respectively. Plasma insulin concentrations did not differ significantly either prior to (12±2 vs. 11±5 μU/ml) or during insulin infusion (30.4±9.4 vs. 33.7±7.1 μU/ml). Both glucose production and utilization rates, determined with [3-^{3}H]glucose in the postabsorptive state were greater in the diabetic than nondiabetic subjects (2.34±0.13 vs. 1.43±0.08 and 2.49±0.13 vs. 1.51±0.08 mg/kg · min, respectively, $P < 0.001$). During infusion of insulin, despite comparable plasma insulin concentrations and slightly higher glucose concentrations, glucose production (1.33±0.15 vs. 0.13±0.18 mg/kg · min, $P < 0.01$) was greater and glucose utilization (1.93±0.10 vs. 2.67±0.35 mg/kg · min, $P < 0.05$) was less in the diabetic patients, indicating the presence of insulin resistance. Prior to insulin infusion, C peptide (2.0±0.2 vs. 1.9±0.6 ng/ml), glucagon (133±18 vs. 121±18 pg/ml), and GH (1.3±0.1 vs. 1.1±0.1 ng/ml) were similar in the diabetic and nondiabetic groups, respectively. No differences between groups became apparent during insulin infusion (C peptide 1.6±0.2 vs. 1.3±0.4 ng/ml; glucagon 116±15 vs. 110±18 pg/ml; GH 1.5±0.2 vs. 1.5±0.3 ng/ml).

Discussion

Diabetes mellitus is characterized by both preprandial and postprandial hyperglycemia. Although the relationship between hyperglycemia and the long-term complications of diabetes mellitus remains uncertain, presumably goals of therapy include not only normalization of plasma glucose concentration but also normalization of the pattern of glucose metabolism. The current studies demonstrate that after carbohydrate ingestion, excessive endogenous glucose production persists and is accompanied by slight but significant increases in the amounts of ingested glucose reaching the systemic circulation both directly and by way of new glucose formation from meal-derived three-carbon precursors in patients with NIDDM. Because this increased rate of glucose entry is not accompanied by an appropriate increase in glucose uptake, postprandial hyperglycemia results. Both insulin resistance, as evidenced by a failure of insulin to adequately suppress glucose production and stimulate glucose utilization,

and impaired insulin secretion, as demonstrated by a decreased C-peptide response after meal ingestion, presumably contribute to this abnormal pattern of postprandial carbohydrate metabolism.

Other investigators (10–12) have reported increased rates of endogenous glucose release in the postabsorptive state in hyperglycemic patients with NIDDM. Data in the postprandial state are far more limited. Felig et al. (18), using the hepatic venous catheterization technique, have suggested that failure of splanchnic glucose retention is the major factor responsible for postprandial hyperglycemia in NIDDM. Because this technique only measured net splanchnic balance, their conclusion was based on the assumption that endogenous glucose release was not suppressed below preprandial rates after meal ingestion in either diabetic or nondiabetic subjects. However, recent studies using a dual isotope technique indicate that endogenous glucose release is markedly suppressed after carbohydrate ingestion in both nondiabetic animals and humans (2, 3, 5, 32). The current studies demonstrate that postprandial suppression of endogenous glucose production also occurs in patients with NIDDM. The magnitude of this suppression, measured as the percent change from preprandial rates, is equivalent to that observed in nondiabetic subjects. Howver, because the basal rates of glucose production are greater in the diabetic patients, the total amount of glucose released from the liver after meal ingestion also is greater whether calculated as release over the 7 h of observation or as release during the interval that plasma glucose remained above preprandial levels. This results in excessive amounts of glucose being presented to extrahepatic tissues for disposal. In that disposal does not increase appropriately, hyperglycemia ensued.

The pattern of suppression of glucose release also differed in the diabetic and nondiabetic subjects. Coincident with the rapid increase in insulin secretion, suppression of endogenous glucose release was prompt and sustained between 90 and 240 min in the nondiabetic subjects. Subsequently, as the plasma glucose concentration fell toward preprandial levels, endogenous glucose release increased towards basal rates, presumably reflecting postprandial counterregulation (41). In contrast in the diabetic patients, glucose production after meal ingestion decreased slowly and progressively, for 5 h. This excessive endogenous glucose production could not be attributed to excessive circulating glucagon concentrations in the NIDDM patients. Both decreased insulin secretion (as reflected by lower insulin and C peptide concentrations) and decreased insulin action (as reflected by impaired suppression of glucose production during the euglycemic clamp) presumably contributed to the excessive postprandial endogenous glucose release in the diabetic patients. However, because the portal insulin and plasma glucose concentrations differed during the hyperinsulinemic clamp and after glucose ingestion, the quantitative contribution of alterations in insulin secretion and insulin action cannot be determined. Of interest, the pattern of suppression of endogenous glucose release observed in the current study of patients with NIDDM is similar to that previously reported in C peptide-deficient patients with insulin-dependent diabetes mellitus (3).

Several investigators have reported that gluconeogenesis is increased in patients with NIDDM in the postabsorptive state (19–21). Furthermore, recent data indicate that a substantial portion of the glycogen synthesized after glucose ingestion originates via the indirect pathway entailing gluconeogenesis from three-carbon precursors rather than from direct hepatic uptake of glucose (7, 42). Taken together, these observations are consistent with the hypothesis that increased rates of new glucose formation from meal-derived three-carbon precursors lead to an increased rate of postprandial endogenous glucose production in diabetic patients. To test this hypothesis, we measured the amount of glucose entering the systemic circulation in which the ^{14}C had been randomized from the sixth to the first position of glucose. Because the ingested glucose was labeled with ^{14}C in the sixth position, the presence of ^{14}C in the first position indicated that such glucose had been metabolized to and resynthesized from meal-derived three-carbon precursors. Assuming equivalent randomization of ^{14}C to the 1, 2, 5, and 6 positions (31, 32), a qualitative estimate of glucose derived via this pathway can be obtained. Using this method, our findings indicate that although systemic new glucose appearance from meal-derived three-carbon precursors was increased approximately threefold in diabetic subjects (3 vs. 1 g), its contribution to their excessive systemic postprandial endogenous glucose release was minimal. Because radioactivity in glycogen was not measured, this does not preclude a substantial difference between groups in glycogen synthesis via this pathway.

It should be emphasized however that in this, as well as in all other in vivo techniques, intrahepatic specific activities of glucose and gluconeogenic precursors are not known (43). Therefore, all in vivo methods provide a qualitative rather than a quantitative estimate of gluconeogenesis (43). Differences among peripheral, portal venous, and intrahepatic specific activities after carbohydrate ingestion preclude the use of the product to precursor relationship normally employed to calculate gluconeogenic rates during systemic infusion of either labeled gluconeogenic precursors or bicarbonate. Inasmuch as in the current experiments, the meal was labeled with [^{14}C]glucose, intestinal and hepatic metabolism of the radioactive and nonradioactive ingested glucose presumably was equivalent. However, because this technique primarily measures new glucose formation from meal-derived precursors, it does not rule out a concomitant increase in the rate of gluconeogenesis from systemic precursors. Hetenyi et al. (44) have suggested a correction factor of approximately 2.2 for dilution of labeled by unlabeled precursors in the oxaloacetate pool during gluconeogenesis. Because this correction factor has only been validated in postabsorptive animals under steady-state conditions, its applicability to humans after meal ingestion remains uncertain. Nevertheless, even invoking such a correction factor, new glucose formation from meal-derived three-carbon precursors still represents a small fraction of total endogenous glucose release in either the diabetic or nondiabetic subjects.

After carbohydrate ingestion, virtually all of the meal-derived glucose eventually reached the systemic circulation over the 7-h study period in the diabetic and nondiabetic subjects. This finding in the nondiabetic subjects is consistent with previous reports (2, 3). However, the amount of meal glucose reaching the systemic circulation during the period of postprandial hyperglycemia was slightly but significantly greater in the diabetic than nondiabetic subjects (622±19 vs. 521±15 mg/kg·min or ~46 vs. 52 g). These results suggest negligible initial hepatic uptake in the diabetic patients compared to ~8% in the nondiabetic patients. The estimate in nondiabetic subjects is consistent with that of previous investigators (2, 3). However, as discussed by Radzuik et al. (2), assuming that recirculating glucose and meal derived glucose are present in the portal vein in a ratio of 5:1, an initial hepatic clearance of 4 g more of meal

glucose is consistent with a 20-g greater total hepatic glucose uptake in the nondiabetic than diabetic patients. However, this estimate must be tempered by the fact that the [^{14}C]glucose used to label the meal could be incorporated into and released from glycogen during the long postabsorptive period. Such "cycling" through glycogen would lead to an underestimate of first-pass hepatic glucose clearance.

The potential influence of the choice of isotope of glucose employed to trace the systemic entry of glucose on the measurements of glucose flux rate in the diabetic patients must be considered. In the dual isotope technique the same isotope is used to estimate the total and meal-derived systemic glucose appearance. Therefore any error inherent in the systemic tracer wil be introduced into measurement of both of these parameters. There is less concern regarding estimates of endogenous glucose because it is calculated by subtracting the meal-derived from the total appearance rate. [2-^{3}H]glucose was used to trace the rate of systemic glucose appearance in the current study. This isotope was chosen because it does not retain its label during uptake and release of glucose from glycogen (45). However, it possesses the disadvantage that it can be detritiated during flux between glucose-6-phosphate and fructose-6-phosphate, a process frequently referred to as futile cycling. Although alternative isotopes such as [3-^{3}H]glucose, [6-^{3}H]glucose, and [6-^{14}C]glucose are not detritiated in the glucose/glucose-6-phosphate cycle, they can be incorporated into and released from glycogen without losing their radioactive label. If this process is active, the latter isotopes will result in an underestimate of both glucose appearance and disappearance. This may be a particular problem in the postprandial state because the outer layers of glycogen may be preferentially labeled at a time when glycogen synthesis and glycogenolysis are occurring simultaneously (46).

In the current study, the greater postprandial endogenous glucose release in the diabetic patients could be entirely ascribed to the greater basal glucose production rate in the diabetic patients. Preprandial production rates determined with [2-^{3}H]glucose were greater than those determined with [3-^{3}H]glucose in both diabetic and nondiabetic subjects indicating the presence of futile cycling, consistent with the report of Efendic et al. (47). However, the greater preprandial glucose production rates in the diabetic patients than in nondiabetic subjects were not due to glucose/glucose-6-phosphate cycling because it was documented independently with both [2-^{3}H]- and [3-^{3}H]glucose. Postprandial glucose turnover rates determined with [2-^{3}H]- and [3-^{3}H]glucose were essentially the same in the diabetic patients restudied using a simultaneous infusion of both isotopes. These studies suggest that both futile cycling and cycling through glycogen were minimal in the diabetic patients after carbohydrate ingestion.

In addition to excessive rates of postprandial systemic glucose entry, a lack of an appropriate postprandial increase in glucose uptake was evident in the diabetic patients. Thus, there was a smaller incremental response above basal in both total body (isotopically determined) and forearm glucose uptake. On the one hand, the demonstration by the hyperinsulinemic clamp study that glucose uptake, at insulin concentrations similar to those present after meal ingestion, was decreased in the diabetic subjects suggests that insulin resistance contributed to the impaired postprandial glucose disposal. On the other hand the failure of an appropriate increase in insulin secretion as indicated by the blunted C-peptide response above baseline was also clearly evident in the diabetic patients. Whether impaired glucose mediated uptake by both insulin-dependent and noninsulin-dependent tissues was also present cannot be assessed from the current experiments.

In contrast to the decreased incremental response in glucose uptake, the absolute rate of glucose uptake measured either isotopically or by forearm catheterization (48) after meal ingestion was not decreased in the diabetic patients. Of interest, the magnitude of differences in glucose uptake between the diabetic and nondiabetic subjects differed when assessed isotopically or by forearm catheterization. This discrepancy may reflect the pattern of intracellular metabolism of the [2-^{3}H]glucose. Glucose uptake measured with [2-^{3}H]glucose primarily assesses transport and phosphorylation (47) whereas glucose uptake measured with forearm catheterization reflects irreversible glucose extraction. The similar postprandial rates of glucose utilization measured with [2-^{3}H] and [3-^{3}H] glucose (an isotope of glucose that is not detritiated in the glucose/glucose-6-phosphate cycle) in the diabetic patients studied with both isotopes argue against futile cycling as an explanation. The cause for the quantitative differences in the isotopic and forearm data are unknown. In contrast to the postprandial state, glucose uptake in the preprandial state (measured in all subjects) not only was higher when assessed with both [2-^{3}H]glucose and [3-^{3}H]glucose but also when measured by forearm glucose catheterization. The observation of increased rates of glucose utilization in the hyperglycemic patients, is consistent with the suggestion that the mass action effect of glucose on glucose uptake compensates for the presence of insulin resistance and/or insulin deficiency (12).

In summary, the current studies demonstrate that the proximal cause of postprandial hyperglycemia in patients with NIDDM is an excessive rate of systemic glucose appearance. Despite the presence of hepatic insulin resistance and decreased insulin secretion, percent suppression of hepatic glucose production is comparable in diabetic and nondiabetic subjects, presumably owing to the compensatory effect of hyperglycemia in the former. However, because preprandial rates are greater, the absolute rate of endogenous glucose release is excessive in diabetic patients after carbohydrate ingestion. Only a minor part of this excessive release can be accounted for by new glucose formation from three-carbon precursors. Postprandial hyperglycemia is further exacerbated by a lack of an appropriate increase in glucose uptake in diabetic patients. Thus, as has been proposed for fasting hyperglycemia, excessive hepatic glucose release and impaired glucose uptake plays a central role in production of postprandial hyperglycemia in patients with NIDDM.

Acknowledgments

The excellent technical assistance of J. King, S. Coman, and D. Rademacher and the superb editorial assistance of K. Welch are gratefully acknowledged. This work was supported in part by grants AM-29953 and RR-00585 from the U.S. Public Health Service, Squibb-Novo, Inc., and the Mayo Foundation. Dr. P. Bell is supported in part by a Fulbright Scholarship.

References

1. Felig, P., J. Wahren, and R. Hendler. 1975. Influence of oral glucose ingestion on splanchnic glucose and gluconeogenic substrate metabolism in man. *Diabetes.* 24:468–475.

2. Radziuk, J., T. McDonald, D. Rubenstein, and J. Dupre. 1978. Initial splanchnic extraction of ingested glucose in normal man. *Metab. Clin. Exp.* 27:657–669.

3. Pehling, G., P. Tessari, J. Gerich, M. Haymond, F. Service, and R. Rizza. 1984. Abnormal carbohydrate disposition in insulin-dependent diabetes: relative contributions of endogenous glucose production and initial splanchnic uptake and effect of intensive insulin therapy. *J. Clin. Invest.* 74:995–991.

4. Katz, L. D., M. D. Glickman, S. Rapoport, E. Ferrannini, and R. A. DeFronzo. 1983. Splanchnic and peripheral disposal of oral glucose in man. *Diabetes.* 32:675–679.

5. Ferrannini, E., O. Bjorkman, G. A. Reichard, A. Pilo, M. Olsson, J. Wahren, and R. A. DeFronzo. 1985. The disposal of an oral glucose load in healthy subjects: a quantitative study. *Diabetes.* 34:580–588.

6. Shikama, H., and M. Ui. 1978. Glucose load diverts hepatic gluconeogenic product from glucose to glycogen. *Am. J. Physiol.* 235:E354–360.

7. Radzuik, J. 1982. Sources of carbon in hepatic glycogen synthesis during absorption of oral glucose load in humans. *Fed. Proc.* 41:110–116.

8. Newgard, C., L. Hirsch, D. Foster, and J. McGarry. 1983. Studies on the mechanism by which exogenous glucose is converted into liver glycogen in the rat. *J. Biol. Chem.* 13:8046–8052.

9. Forbath, N., and G. Hetenyi. 1966. Glucose dynamics in normal subjects and diabetic patients before and after a glucose load. *Diabetes.* 15:778–789.

10. Bowen, H., and J. Moorhouse. 1973. Glucose turnover and disposal in maturity-onset diabetes. *J. Clin. Invest.* 52:3033–3045.

11. Best, J., R. Judzewitsch, M. Pfeifer, J. Beard, J. Kolter, and D. Porte, Jr. 1982. The effect of chronic sulfonylurea therapy on hepatic glucose production in non-insulin dependent diabetes. *Diabetes.* 31:333–338.

12. Revers, R., R. Fink, J. Griffin, J. Olefsky, and O. Kolterman. 1984. Influence of hyperglycemia on insulin's in vivo effects in type II diabetes. *J. Clin. Invest.* 73:664–672.

13. Himsworth, H., and R. Kerr. 1939. Insulin sensitive and insulin-insensitive types of diabetes mellitus. *Clin. Sci.* 4:119–152.

14. DeFronzo, R., D. Diebert, R. Hendler, and P. Felig. 1979. Insulin sensitivity and insulin binding to monocytes in maturity-onset diabetes. *J. Clin. Invest.* 63:939–946.

15. Kolterman, O., R. Gray, J. Griffin, P. Burstein, J. Insel, J. Scarlett, and J. Olefsky. 1981. Receptor and postreceptor defects contribute to the insulin resistance in non-insulin dependent diabetes mellitus. *J. Clin. Invest.* 68:957–965.

16. Rizza, R., L. Mandarino, and J. Gerich. 1981. Mechanism and significance of insulin resistance in non-insulin dependent diabetes mellitus. *Diabetes.* 30:990–995.

17. Reaven, G., R. Bernstein, B. Davis, and J. Olefsky. 1976. Nonketotic diabetes mellitus: insulin deficiency or insulin resistance? *Am. J. Med.* 60:80–88.

18. Felig, P., J. Wahren, and R. Hendler. 1978. Influence of maturity-onset diabetes on splanchnic glucose balance after oral glucose ingestion. *Diabetes.* 27:121–126.

19. Wahren, J., P. Felig, E. Cerasi, and R. Luft. 1972. Splanchnic and peripheral glucose and amino acid metabolism in diabetes mellitus. *J. Clin. Invest.* 51:1870–1878.

20. Chochinov, R., H. Bowen, and J. Moorhouse. 1978. Circulating alanine disposal in diabetes mellitus. *Diabetes.* 27:420–426.

21. Hall, S., J. Braaten, J. McKendry, T. Bolton, D. Foster, and M. Berman. 1979. Normal alanine-glucose relationships and their change in diabetic patients before and after insulin treatment. *Diabetes.* 28:737–745.

22. Jackson, R., N. Peters, U. Advani, G. Perry, J. Rogers, W. Brough, and T. Pilkington. 1973. Forearm glucose uptake during the oral glucose tolerance test in normal subjects. *Diabetes.* 22:442–458.

23. Rizza, R., L. Mandarino, and J. Gerich. 1981. Dose-response characteristics for effects of insulin on production and utilization of glucose in man. *Am. J. Physiol.* 240:E630–639.

24. Coles, D., K. Cooper, R. Mottram, and J. Occleshaw. 1958. The source of blood samples withdrawn from deep forearm veins via catheters passed upstream from the median cubital vein. *J. Physiol. (Lond).* 142: 323–328.

25. Figar, S. 1959. Some basic deficiencies of the plethysmographic method and possibilities of avoiding them. *Angiology.* 10:120–125.

26. Hyman, C., D. Burnap, and S. Figar. 1963. Bilateral differences in forearm blood flow as measured with capacitance plethysmography. *J. Appl. Physiol.* 18:997–1002.

27. Somogyi, M. 1945. Determination of blood sugar. *J. Biol. Chem.* 160:69–73.

28. Nakagawa, S., H. Nakayam, T. Sasaki, K. Yoshima, Y. Yu, K. Shinozaki, S. Aoki, and K. Mashimo. 1973. A simple method for the determination of serum free insulin levels in insulin treated patients. *Diabetes.* 22:590–600.

29. Heding, L. 1975. Radioimmunological determination of human C-peptide in serum. *Diabetologia.* 11:541–548.

30. Faloona, G., and R. Unger. 1974. Glucagon. *In* Methods of hormone Radioimmunoassay. B. Jaffe and H. Behrman, editors. Academic Press, Inc., New York. 317–330.

31. Reichard, G., N. Moury, N. Hochella, A. Patterson, and S. Weinhouse. 1963. Quantitative estimation of the Cori cycle in the human. *J. Biol. Chem.* 238:495–501.

32. Steele, R., C. Bjerknes, I. Rothgeb, and N. Altszuler. 1968. Glucose uptake and production during the oral glucose tolerance test. *Diabetes.* 17:415–421.

33. Kalhan, S., S. Savin, and P. Adam. 1977. Estimation of glucose turnover with stable tracer glucose-1-^{13}C. *J. Lab. Clin. Med.* 89:285–294.

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

35. DeBodo, R., R. Steele, N. Altszuler, A. Dunn, and J. Bishop. 1963. On the hormonal regulation of carbohydrate metabolism: studies with C^{14}-glucose. *Recent Prog. Horm. Res.* 19:445–488.

36. Cherrington, A., W. Lacy, and J. Chiasson. 1978. Effect of glucagon on glucose production during insulin deficiency in the dog. *J. Clin. Invest.* 62:664–678.

37. Dillon, R. 1965. Importance of hematocrit in interpretation of blood sugar. *Diabetes.* 14:672–678.

38. Issekutz, B. 1977. Studies on hepatic glucose cycles in normal and methylprednisolone-treated dogs. *Metab. Clin. Exp.* 26:157–170.

39. Peake, G. 1974. Growth hormone. *In* Methods of Hormone Radioimmunoassay. J. Jaffe and H. Behrman, editors. Academic Press, Inc. New York. 103–121.

40. Zar, J. 1974. Biostatistical Analysis. Prentice Hall, Inc., Englewood Cliffs, NJ.

41. Tse, T., W. Clutter, S. Shah, and P. Cryer. 1983. Mechanisms of postprandial glucose counterregulation in man: physiologic roles of glucagon and epinephrine vis-a-vis insulin in the prevention of hypoglycemia late after glucose ingestion. *J. Clin. Invest.* 72:278–286.

42. Katz, J., and J. McGarry. 1984. The glucose paradox: is glucose a substrate for liver metabolism. *J. Clin. Invest.* 74:1901–1909.

43. Brosnan, J. 1982. Pathways of carbon flux in gluconeogenesis. *Fed. Proc.* 41:91–95.

44. Hetenyi, G. 1982. Correction for the metabolic exchange of ^{14}C for ^{12}C atoms in the pathway of gluconeogenesis in vivo. *Fed. Proc.* 41: 104–109.

45. Katz, J., and R. Rognstad. 1976. Futile cycles in the metabolism of glucose. *Curr. Top. Cell. Regul.* 10:237–289.

46. Devos, P., and H.-G. Hers. 1979. A molecular order in the synthesis and degradation of glycogen in the liver. *Eur. J. Biochem.* 99:161–167.

47. Efendic, S., A. Wajngot, and M. Vranic. 1985. Increased activity of the glucose cycle in liver: early characteristic of type 2 diabetes. *Proc. Natl. Acad. Sci. USA.* 82:2965–2969.

48. Jackson, R., G. Perry, J. Rogers, U. Advani, and T. Pilkington. 1973. Relationship between the basal glucose concentration, glucose tolerance and forearm glucose uptake in maturity-onset diabetes. *Diabetes.* 22:751–761.