Influence of Obesity and Type 2 Diabetes on Gluconeogenesis and Glucose Output in Humans

A Quantitative Study

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The contribution of gluconeogenesis (GNG) to endogenous glucose output (EGO) in type 2 diabetes is controversial. Little information is available on the separate influence of obesity on GNG. We measured percent GNG (by the ^2H_2O technique) and EGO (by 6,6-[^{2}H]glucose) in 37 type 2 diabetic subjects (9 lean and 28 obese, mean fasting plasma glucose [FPG] 8.3 ± 0.3 mmol/l) and 18 control subjects (6 lean and 12 obese) after a 15-h fast. Percent GNG averaged 47 ± 5% in lean control subjects and was significantly increased in association with both obesity ($P < 0.01$) and diabetes ($P = 0.004$). By multivariate analysis, percent GNG was independently associated with BMI (partial $r = 0.27$, $P < 0.05$, with a predicted increase of 0.9% per BMI unit) and FPG (partial $r = 0.44$, $P = 0.0009$, with a predicted increase of 2.7% per mmol/l of FPG). In contrast, EGO was increased in both lean and obese diabetic subjects (15.6 ± 0.5 μmol · min^{-1} · kg^{-1} of fat-free mass, $n = 37$, $P = 0.002$) but not in obese nondiabetic control subjects (13.1 ± 0.7, NS) as compared with lean control subjects (12.4 ± 1.4). Consequently, gluconeogenic flux (percent GNG × EGO) was increased in obesity ($P = 0.01$) and markedly elevated in diabetic subjects ($P = 0.0004$), whereas glycogenolytic flux was reduced only in association with obesity ($P = 0.05$). Fasting plasma glucagon levels were significantly increased in diabetic subjects ($P < 0.05$) and positively related to EGO, whereas plasma insulin was higher in obese control subjects than lean control subjects ($P = 0.05$) and unrelated to measured glucose fluxes. We conclude that the percent contribution of GNG to glucose release after a 15-h fast is independently and quantitatively related to the degree of overweight and the severity of fasting hyperglycemia. In obese individuals, reduced glycogenolysis ensures a normal rate of glucose output. In diabetic individuals, hyperglucagonemia contributes to inappropriately elevated rates of glucose output from both GNG and glycogenolysis. ***Diabetes*** **49:1367–1373, 2000**

Patients with type 2 diabetes typically show an increase in endogenous glucose output (EGO) (1–3). Several observations indicate that gluconeogenesis (GNG) is increased in type 2 diabetes. First, there is an increased protein turnover and hence release of gluconeogenic amino acids. Second, in obese patients with type 2 diabetes, the increased fat mass and rate of lipolysis contribute substrate (glycerol) (4,5) and activation (free fatty acids) (6,7) for GNG. Third, the net uptake of all gluconeogenic precursor substrates by the liver is increased. Using splanchnic catheterization, Felig et al. (8) estimated that in diabetic subjects, conversion into glucose of gluconeogenic precursors could represent 30% of splanchnic glucose output versus 20% in lean nondiabetic subjects (8). Although net splanchnic substrate uptake cannot account for the whole of GNG, this finding is nevertheless compatible with enhanced GNG in diabetes. Finally, tracer studies have shown that in obese type 2 diabetic patients, lactate and alanine production is increased 3-fold, and conversion into glucose is increased 3.5-fold (9). In another study using labeled glucose, the rate of the Cori cycle also was found to be increased in obese Pima Indians with type 2 diabetes (10). Using ^{13}C nuclear magnetic resonance (NMR), Magnusson et al. (11) found increased GNG in type 2 diabetic patients after prolonged (23 h) fasting. Other studies however have not confirmed the finding of enhanced GNG in diabetes (12,13). Multiple reasons may explain the variability of results in human studies: *1*) GNG has been measured in case-control studies involving small numbers of subjects; *2*) when GNG was measured with the use of tracer techniques based on the infusion of carbon-labeled precursors of glucose, dilution of the label in the oxaloacetate pool (in the case of lactate, pyruvate, and alanine) and partial tracing of the pathway (in the case of glycerol) (14) have resulted in uncertain estimates (possibly underestimates) of GNG; *3*) estimates of GNG made from the incorporation of ^{14}C from [2-^{14}C]acetate into glucose and hydroxybutyrate, presumed to overcome those limitations, are inaccurate because of extensive metabolism of acetate in tissues other than liver (15) and dilution of the specific activity of hydroxybutyrate formed in the liver

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C5, carbon 5; EGO, endogenous glucose output; FFM, fat-free mass; FPG, fasting plasma glucose; G6P, glucose-6-phosphate; GAP, glyceraldehyde-3-phosphate; GCMS, gas chromatography/mass spectrometry; GNG, gluconeogenesis; HPLC, high-performance liquid chromatography; MIDA, mass isotopomer distribution analysis; NMR, nuclear magnetic resonance; PEP, phosphoenolpyruvate; WHR, waist-to-hip ratio.

by unlabeled hydroxybutyrate formed in muscle (9,11); and *4*) the use of mass isotopomer distribution analysis (MIDA) with [2-$^{13}C_1$]glycerol or [U-^{13}C]glucose has limitations (16,17).

Thus, whereas the preponderance of evidence supports enhanced GNG in human diabetes, the role of factors such as sex, age, obesity, and degree of glycemic control has not been examined. This result prompted us to undertake the present study, in which GNG was estimated by the ^2H_2O technique (18,19) and EGO was measured by the tracer glucose technique. A large number of lean and obese patients with type 2 diabetes and nondiabetic control subjects in the overnight fasted state were studied.

RESEARCH DESIGN AND METHODS

Subjects. The study population consisted of 55 women and men with a mean age of 53 years (range 22–69) and a mean BMI of 29.4 kg/m^2 (range 22.6–43.6). Study subjects were recruited from the diabetes and obesity clinics on a consecutive basis, not prospectively. By defining obesity as a BMI ≥27 kg/m^2, 40 study subjects were obese and 15 were nonobese. Among them, 28 and 9 subjects, respectively, had previously diagnosed type 2 diabetes; the others had normal oral glucose tolerance (20). The clinical and metabolic characteristics of the 4 groups into which the population was divided are given in Table 1. Except for diabetes, all subjects were free of major diseases as determined by medical history, physical examination, and routine blood chemistry. Diabetic subjects were on treatment with diet alone or in combination with oral hypoglycemic agents (sulfonylureas, metformin, or both); oral agents were withdrawn at least 4 weeks before the study. Nondiabetic subjects were not taking any medication known to affect glucose metabolism. The study protocol was approved by the Institutional Ethics Committee, and each subject gave his/her informed written consent to participate.

Protocol. Subjects were asked to drink ^2H_2O (5 g/kg total body water, which was taken to be 55 and 45% of body weight in men and women, respectively) at 10:00 P.M. the night before the study (~2 h after a light dinner composed of 50% carbohydrate, 15% protein, and 35% fat). To minimize vertigo, subjects were instructed to sip the ^2H_2O dose over a period of 30 min and to rest in bed thereafter. Because all study subjects were ambulatory and some were driving to the hospital the next morning for the study, the ^2H_2O load was not split between a late evening and an early morning dose to avoid interference with sleep patterns and to avoid vertigo while driving. Six subjects (10%) reported moderate to severe vertigo and nausea, subsiding within 1 h; upon questioning, half of the subjects reported transient mild vertigo.

After the overnight fast, during which subjects were instructed not to drink, the study was initiated in the morning at 9:00 A.M. Waist-to-hip ratio (WHR) was determined by measuring the waist circumference at the narrowest part of the torso and the hip circumference in a horizontal plane at the level of the maximal extension of the buttocks. An indwelling catheter was placed into an antecubital vein for isotope infusion. A second catheter was inserted retrogradely into a wrist vein of the ipsilateral hand, and the hand was placed into a heated box (60°C) to achieve arterialization of venous blood. A primed continuous infusion of 6,6-[^{2}H]glucose (4 mg/kg as the prime, followed by a continuous infusion at a rate of 0.04 mg · min^{-1} · kg^{-1}) was performed for 120 min in nondiabetic subjects. In diabetic subjects, the priming dose was increased in proportion to the increase (above 5 mmol/l) in fasting plasma glucose (FPG) concentrations and was extended to 180 min to ensure tracer equilibration in the presence of reduced plasma glucose clearance (21). Plasma samples for the determination of 6,6-[^{2}H]glucose enrichment, plasma insulin, and glucagon concentrations were obtained before starting the tracer infusion and every 10 min during the last 20 min of the infusion. For the determination of carbon 5 (C5) enrichment, plasma samples were obtained on a separate day before the study to measure natural plasma glucose enrichment and at the end of the 6,6-[^{2}H]glucose infusion on the day of the study. The mean duration of fasting for the whole study group was 15.4 ± 0.2 h. In 32 of 55 subjects (10 control subjects and 22 diabetic patients, BMI 30.8 ± 1.5 vs. 28.8 ± 0.6 kg/m^2, NS), a sample for the determination of C5 enrichment was also obtained after 12 h of fasting (i.e., before starting the 6,6-[^{2}H]glucose infusion).

Analytical methods. ^2H_2O and 6,6-[^{2}H]glucose were purchased from Mass Trace (Woburn, MA). Glucose concentration was determined by the glucose oxidase method (Beckman II Glucose Analyzer; Beckman, Fullerton, CA). Plasma insulin and glucagon concentrations were measured by radioimmunoassay (Linco Research, St. Louis, MO). Serum HbA_{1c} concentrations were measured by high-performance liquid chromatography (HPLC) (Menarini HA-8140; Menarini Diagnostics, Florence, Italy).

Plasma glucose enrichment was determined by gas chromatography/mass spectrometry (GCMS) on a Hewlett Packard GC 5890/MS 5972 (Hewlett Packard, Palo Alto, CA) equipped with a 30-m capillary column, as described by Wolfe (22). Briefly, the plasma sample was deproteinized using equal volumes of 0.3 N $ZnSO_4$ and 0.3 N $Ba(OH)_2$ (Somogyi procedure), and the supernatant was passed through a mixed column of AG 1-X8 in the formate form and AG 50W-X8 in the H^+ form. To measure glucose enrichment due to 6,6-[^{2}H]glucose, the dried sample was derivatized using acetic anhydride:pyridine (1:1) to form pentaacetate glucose. The sample was then dried again and dissolved with ethylacetate for injection into the GCMS. The fragments 200, 201, and 202 were monitored, and enrichment was calculated as the ratio of 202/200. To correct for incorporation into glucose of ^{2}H originating from the administered ^2H_2O, the 202/200 ratio obtained at the end of the 6,6-[^{2}H]glucose infusion was corrected for the corresponding ratio measured in the plasma sample collected before the start of the 6,6-[^{2}H]glucose infusion. A further correction was introduced to account for the possible incorporation of deuterium from ^2H_2O into glucose occurring between the beginning and the end of the 6,6-[^{2}H]glucose infusion (overlapping spectra correction, as per Eq. 5 in the article by Wolfe [22]).

The pattern of ^{2}H incorporation into plasma glucose after ^2H_2O ingestion was determined according to the method developed by Landau and associates (18,19). Briefly, the fraction of glucose produced via GNG from all precursors can be quantified from the ratio of ^{2}H enrichment of C5 to that of water. The

TABLE 1
Clinical and metabolic characteristics

	Control subjects			Diabetic subjects		
	Lean	*P**	Obese	Lean	*P**	Obese
n	6		12	9		28
Sex (M/F)	2/4	NS	6/6	8/1†	NS	18/10
Age (years)	44 ± 5	NS	42 ± 4	59 ± 2†	NS	58 ± 2†
BMI (kg/m^2)	25.7 ± 0.5	0.001	32.8 ± 1.2	25.4 ± 0.4	0.0001	30.1 ± 0.4
FFM (kg)	50 ± 3	NS	57 ± 2	51 ± 2	NS	54 ± 1
Fat mass (%)	33 ± 1	0.01	39 ± 1	29 ± 1	0.0001	36 ± 1
Waist circumference (cm)	81 ± 2	0.004	102 ± 3	92 ± 2	0.0004	101 ± 1
WHR (cm/cm)	0.80 ± 0.02	NS	0.86 ± 0.03	0.92 ± 0.01†	NS	0.93 ± 0.01†
Fasting plasma glucose (mmol/l)	5.12 ± 0.15	NS	5.11 ± 0.16	8.18 ± 0.34†	NS	8.47 ± 0.37†
Diabetes duration (years)	—	—	—	8 ± 2	NS	8 ± 1
HbA_{1c} (%)	—	—	—	7.5 ± 0.3	NS	7.4 ± 0.3
Fasting plasma insulin (pmol/l)	47 ± 5	0.03	65 ± 5	57 ± 4	NS	72 ± 7
Fasting plasma glucagon (ng/l)	81 ± 9	NS	65 ± 6	102 ± 17	NS	86 ± 6†

Data are means ± SE, unless otherwise indicated. *For the difference between lean and obese subjects. †$P < 0.05$ for the difference between control subjects and diabetic subjects, adjusted for sex and age by multiple regression.

TABLE 2
Glucose output and its components

	Control subjects			Diabetic subjects		
	Lean	*P**	Obese	Lean	*P**	Obese
GNG (%)	47 ± 5	0.006	62 ± 2	64 ± 5†	NS	68 ± 2
Total glucose output (μmol · min^{-1} · kg^{-1} FFM)	12.4 ± 1.4	NS	13.1 ± 0.7	16.7 ± 0.9†	NS	15.2 ± 0.6†
Gluconeogenic flux (μmol · min^{-1} · kg^{-1} FFM)	5.6 ± 0.6	0.01	8.3 ± 1.0	10.8 ± 1.0†	NS	10.3 ± 0.5†
Glycogenolytic flux (μmol · min^{-1} · kg^{-1} FFM)	6.7 ± 1.4	0.05	4.8 ± 0.4	5.9 ± 0.8	NS	4.9 ± 0.4

Data are means ± SE or *P*. *For the difference between lean and obese subjects; †$P < 0.05$ for the difference between control and diabetic subjects.

precursor of the hydrogen bound to C5 of glucose is the hydrogen bound to C2 of glyceraldehyde-3-phosphate (GAP). Hydrogen equilibrates with the hydrogen of body water in the isomerization of GAP with dihydroxyacetone phosphate, an intermediate in the conversion of glycerol to glucose, and binds in the hydration of phosphoenolpyruvate (PEP) (formed in the conversion of pyruvate to glucose). Because there is no binding of hydrogen from body water to C5 of the glucose formed during glycogen breakdown, enrichment at C5 in blood glucose versus water reflects the fractional contribution of total GNG, i.e., from both PEP precursors and glycerol.

Plasma samples were first deproteinized by the Somogyi procedure. The supernatant was then passed through a mixed column of AG 1-X8 in the formate form and AG 50W-X8 in the H^+ form; the eluate was dried in a Speed-Vac (Savant, France). Samples were then reconstituted with 220 μl distilled water and injected into a high-performance liquid chromatograph (Waters) for further purification. Deuterium enrichment at C5 was obtained by converting glucose to xylose by the removal of carbon in position 6. The xylose was purified by HPLC, the C5 group was cleaved by oxidation with periodic acid, and the formaldehyde was collected by distillation. The formaldehyde was incubated with ammonia overnight: in the presence of ammonia, 6 molecules of formaldehyde reacted to form a molecule of hexamethylenetetramine. This step is used to increase the sensitivity of the method. Enrichment of hexamethylenetetramine obtained from C5 was determined by GCMS by monitoring peaks of mass 140 and 141. Precision and accuracy of C5 measurements were determined by running 2 samples (each in triplicate) for each of 5 dilutions of uniformly labeled deuterated glucose (Mass Trace) ranging in enrichment from 0.1 to 0.5%. Over this range, intrasample precision (percent variation coefficient) was 0.7 to 5.2%, whereas intersample precision varied from 1% at 0.5% enrichment to 13% at 0.1% enrichment. Accuracy was determined by regressing observed on expected enrichment; the intercept was 5×10^{-5}, and the slope was 1.027, with an r^2 value of 0.996.

Water enrichment in the body water pool was monitored by reacting a sample of plasma or urine with calcium carbide (CaC_2), thereby obtaining acetylene (C_2H_2), the enrichment of which was then determined by GCMS by monitoring peaks of mass 26 and 27 (23). All samples were run through the GCMS processing in duplicate or triplicate.

Data analysis. In all subjects, both plasma glucose concentrations and 6,6-[^{2}H]glucose enrichment were stable during the last 20 min of tracer infusion. Therefore, total EGO was calculated as the ratio of the 6,6-[^{2}H]glucose infusion rate to the plasma 6,6-[^{2}H]glucose enrichment (mean of 3 determinations). The percent contribution of total GNG to plasma glucose was calculated as the ratio of the enrichments C5/^2H_2O in each study subject. Gluconeogenic flux was calculated by multiplying percent GNG by EGO. The glycogenolytic flux was obtained as the difference between EGO and the gluconeogenic flux. Fat-free mass (FFM) was estimated with the use of Hume's formula in its sex-specific version (24).

Data are given as the mean ± SE. Comparison of mean group values was performed by the unpaired *t* test; when appropriate, these comparisons were adjusted for continuous variables by regression analysis. Paired means were compared by Wilcoxon's signed rank test. Simple and partial correlation analyses were used to estimate associations among continuous variables in the whole data set.

RESULTS

The diabetic subjects were older and more often men in comparison with nondiabetic subjects but were well matched for BMI (Table 1). The sex-adjusted WHR was significantly increased in association with diabetes. Degree of fasting hyperglycemia on the day of study, overall glycemic control (i.e., serum HbA_{1c} levels), and known duration of diabetes were not significantly different between lean and obese diabetic subjects. FPG did not change significantly in nondiabetic subjects over the 2 h of tracer glucose infusion (5.3 ± 0.1 vs. 5.1 ± 0.1 mmol/l, $P = 0.08$); in contrast, in diabetic subjects, FPG fell from 9.5 ± 0.4 to 8.4 ± 0.3 mmol/l (i.e., by 0.05 ± 0.01% per min, $P < 0.0001$) over the 3 h of tracer glucose infusion.

In the whole data set, percent GNG had a variation coefficient of 20%, was virtually identical in men (64 ± 2%, $n = 34$) and women (64 ± 3%, $n = 21$), and was unrelated to age. Percent GNG averaged 47 ± 5% in lean nondiabetic subjects and was significantly increased in both obese and diabetic subjects (Table 2). In the whole data set, percent GNG was directly related to FPG concentration (Fig. 1). By multivariate analysis, percent GNG was independently associated with BMI (partial $r = 0.27$, $P < 0.05$, with a predicted increase of 0.9% per BMI unit) and FPG (partial $r = 0.44$, $P = 0.0009$, with a predicted

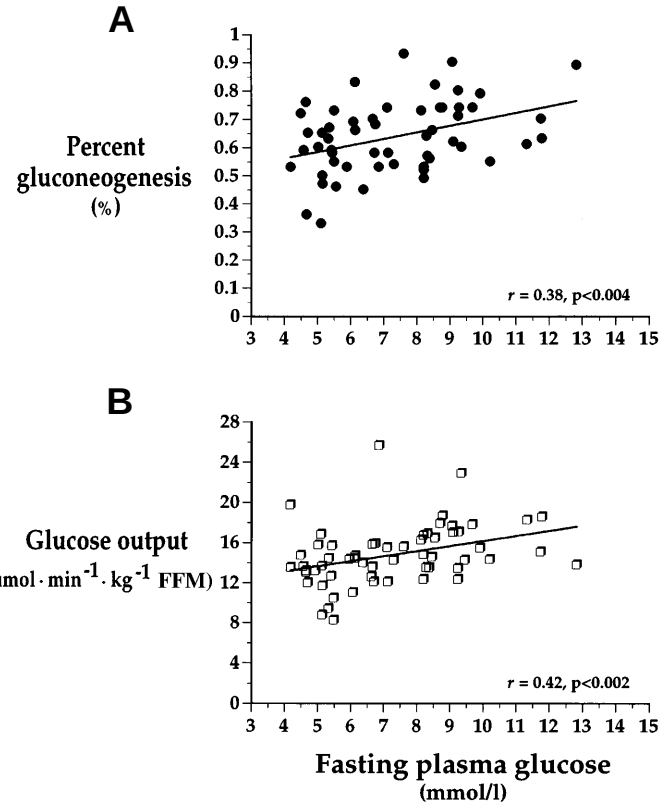

FIG. 1. Linear relationship between FPG and percent GNG (*A*) and EGO (*B*).

increase of 2.7% per millimole per liter of FPG). In the univariate as well as multivariate analysis, neither the waist circumference nor the WHR was associated with percent GNG.

In the 32 BMI-matched subjects for whom measurements were obtained at both 12 and 15 h of fasting, water enrichment was 0.44 ± 0.01% at 12 h and 0.43 ± 0.01% at 15 h, i.e., the water pool was in equilibrium with the tracer water. In these subjects, percent GNG was 48 ± 6% in control subjects and 60 ± 3% in diabetic subjects at 12 h ($P = 0.04$) and 59 ± 5 and 71 ± 2%, respectively, at 15 h ($P = 0.02$).

In the whole study group, EGO had a variation coefficient of 21% and was larger in men than in women (835 ± 24 vs. 708 ± 40 μmol/min, $P < 0.01$). Among the available indexes of body size (body weight and surface area, height, and FFM), EGO was best related to FFM ($r = 0.36$, $P < 0.01$) but was unrelated to indexes of fatness (BMI, fat mass, and percent fat mass). When expressed per kilogram of FFM, EGO was increased in association with diabetes but not obesity (Table 2). Gluconeogenic flux was elevated in obese subjects and even more so in the diabetic patients, whereas glycogenolytic flux was only reduced in the obese nondiabetic group. This pattern of results was unchanged when using BMI and FPG instead of obesity and diabetes (Fig. 1) and was not altered by adjustment for sex and age.

Fasting plasma true insulin levels were significantly raised in obese versus lean control subjects, whereas fasting plasma glucagon concentrations were elevated in diabetic subjects and tended to be lower in the obese subjects (Table 1). Whereas none of the glucose fluxes was significantly associated with plasma insulin levels, both EGO and the gluconeogenic flux were closely related to plasma glucagon in a direct fashion ($r = 0.44$, $P = 0.001$ for both). By multivariate analysis, plasma glucagon and FPG were independently related to GNG (with partial r values of 0.45 and 0.51, respectively), explaining 41% of its observed variability ($P < 0.0001$).

DISCUSSION

Methodology. The measurement of GNG in vivo has been the subject of considerable controversy, mostly because of inaccurate methodology. Whereas techniques based on the incorporation of labeled precursors into plasma glucose underestimate gluconeogenic flux (because of label dilution along the pathway), the ^2H_2O technique measures total GNG (i.e., the sum of PEP and dihydroxyacetone phosphate flux into GAP and, eventually, into glucose-6-phosphate [G6P]) with the use of a few robust assumptions (18,19). The technique has been directly compared with ^{13}C NMR, an independent approach that estimates total GNG by the difference between glucose output and net glycogenolysis (25). The value in our control subjects fasted for 15 h (47 ± 5%) is close to 50 ± 3% that measured in control subjects from another laboratory also using the ^2H_2O technique (26), as well as values of 47 ± 4% after 14 h of fasting and 54 ± 2% between 14 and 16 h of fasting in healthy volunteers, as previously reported by Landau and associates (18,19). By ^{13}C NMR, Petersen et al. (25) estimated GNG at 55 ± 6% from 6 to 12 h of fasting. With the use of [2-$^{13}C_1$]glycerol, by MIDA, Hellerstein et al. (27) estimated the percent GNG in 13 nonobese subjects to average 36 ± 3% after 11 h of fasting. In a study using [U-^{13}C]glucose and MIDA in 6 healthy volunteers, Katz and Tayek (28) reported a value of 41% after 12 h of fasting. The validity of the assumptions made in obtaining the estimates using MIDA has been examined (29,30).

The possibility should be considered that G6P may cycle through glycogen so that labeled glycosyl units from C5-labeled G6P are deposited in glycogen while unlabeled glycosyl units are released by glycogenolysis into glucose. This cycle would result in an underestimation of the contribution of GNG. Evidence shows that glycogen cycling is not significant in fasting normal subjects (19,30). In diabetes, evidence is sparse. However, in fasted alloxan- or streptozotocin-induced diabetic rats, there is negligible incorporation of labeled glucose into hepatic glycogen (31,32). Furthermore, to the extent that the glycosyl units last deposited are first released by glycogenolysis (33), glycogen cycling, if present, would not affect the estimates.

Pathophysiology. The present series included subjects with BMIs of 22.6–43.6 kg/m^2 and FPG concentrations between 4.2 and 12.8 mmol/l who were studied under ordinary living conditions (i.e., ambulatory, on their habitual diet, and with an intact sleep pattern). In the diabetic subjects, glycemic control was fair on average despite antidiabetic drug withdrawal, with no patient having an HbA_{1c} >11.7% after 4 weeks of pharmacological washout. The first major finding was that percent GNG was increased in association with both obesity and diabetes, independently of one another. This result held true whether categorical definitions of obesity and diabetes or surrogate indices (BMI and FPG) were used. From the regression coefficients of multivariate analysis, BMI and FPG predicted increases in percent GNG of 0.9% per BMI unit and 2.7% per mmol/l of fasting glucose above the value of the lean nondiabetic subjects after a 15-h fast.

Previous studies estimating total percent GNG in type 2 diabetes have yielded conflicting results. Thus, percent GNG by ^{13}C NMR (estimated as the difference between glucose output and net glycogenolysis) was found to be significantly increased in 7 diabetic patients (88 vs. 70% of 5 nondiabetic subjects) after 23 h of fasting. In contrast, by using [U-^{13}C]glucose and MIDA, Tayek and Katz (12) reported similar percent contribution of GNG to glucose output in 9 diabetic and 8 nondiabetic subjects. Diraison et al. (13) used [3-^{13}C]lactate and phenylacetate sampling of liver glutamine to assess PEP-based GNG and found lower GNG in 5 diabetic subjects than in 5 nondiabetic subjects fasted overnight.

In the present study, the first study to apply the ^2H_2O technique in a large group of subjects, the sex-, age-, and BMI-adjusted increment in percent GNG in diabetic subjects was 10% (67 vs. 57%) and was linearly related to the degree of fasting hyperglycemia (Fig. 1). If only diabetic subjects with mild fasting hyperglycemia (≤7.8 mmol/l, $n = 13$) or only obese subjects were included in the comparison, the difference in percent GNG between diabetic subjects and control subjects (7–9%) would no longer be statistically significant. Thus, the dual dependence of percent GNG on obesity and diabetes should be taken into account when interpreting data in the literature. The current data provide conclusive evidence that the fraction of plasma glucose that is derived from GNG is increased in patients with type 2 diabetes and quantitatively contributes to their fasting hyperglycemia (Fig. 1). Interestingly, this conclusion agrees with the almost unanimous findings from studies that have estimated minimal GNG by measuring the splanchnic extraction of precursors (8) or the incorporation of labeled lactate/alanine into circulating glucose (2).

With regard to obesity, although Felig et al. (34) reported increased splanchnic uptake of gluconeogenic precursors in

obesity over 25 years ago, only one study has estimated GNG in nondiabetic obese subjects (35) by prelabeling liver glycogen with [U-^{13}C]glucose and then measuring $^{13}CO_2$ and plasma [^{13}C]glucose enrichment and respiratory gas exchange. Percent GNG was found to be significantly higher in 5 obese nondiabetic subjects than in 6 lean nondiabetic subjects. In our series, the sex-, age-, and diabetes-adjusted increase in percent GNG associated with obesity (as defined here) was +7.8% and was directly related to BMI. In contrast, neither the waist circumference nor the WHR was found to bear any relation to GNG or glucose fluxes. It remains to be established whether this finding reflects the fact that the WHR is a poor marker of visceral fat accumulation or whether under the conditions of an overnight fast, GNG is independent of the pattern of fat distribution.

The second major result of our study concerns endogenous glucose fluxes. Over the short time period (2–3 h) of tracer equilibration (during which FPG declines slowly), glucose output roughly equals glucose disappearance (i.e., tissue utilization plus glycosuria, if any exists). The finding in our data that the strongest correlate of EGO was the FFM (and not the fat mass or other measures of adiposity) implies that in the fasting state, glucose utilization exerts an important control on glucose release. Therefore, in men versus women (or in obese vs. lean subjects) in whom lean mass is expanded, a greater demand for glucose is imposed on the glucose-producing organs (liver and kidney) (36). Accordingly, in absolute terms, glucose output was 20% higher in the nondiabetic obese subjects as compared with the lean control subjects—the difference disappearing when lean mass was accounted for (Table 2). On the other hand, and in agreement with most previous measurements (2), total glucose output was increased in the diabetic subjects both in absolute terms and per kilogram of FFM—the increment being roughly proportional to the degree of fasting hyperglycemia (Fig. 1). Identically, plasma glucose disappearance was enhanced, clearly through the mass action effect of the prevailing hyperglycemia because plasma glucose clearance was depressed despite the normal peripheral plasma insulin concentrations (Fig. 2). From the data, it can be calculated that if a diabetic patient has the same fasting glucose clearance as a nondiabetic subject, the same endogenous glucose overproduction would result in an FPG of only 6 mmol/l instead of the 8.4 mmol/l that is actually observed. Thus, although glucose disappearance is increased rather than decreased in absolute terms in diabetic subjects, both glucose overproduction and reduced plasma glucose clearance quantitatively contribute to fasting hyperglycemia.

The gluconeogenic component of total glucose flux was increased in obese subjects and markedly elevated in the diabetic subjects, whereas the glycogenolytic component was significantly reduced only in the obese subjects. Thus, in the obese nondiabetic individual, EGO is maintained at normal levels, i.e., appropriate for the mass of glucose-utilizing tissues, in the face of enhanced GNG by a restrained glycogen breakdown. This compensation is incomplete in the diabetic patient, in whom the larger gluconeogenic flux therefore results in an absolute increase in EGO (Table 2). It is important to stress that in diabetic subjects, gluconeogenic glucose flux exceeded that of the control subjects (by 3 μmol · min^{-1} · kg^{-1} FFM on average), and glycogenolysis was similar (5.1 vs. 5.4 μmol · min^{-1} · kg^{-1} FFM on average), despite the fact that the combination of normal fasting insulin levels and fasting

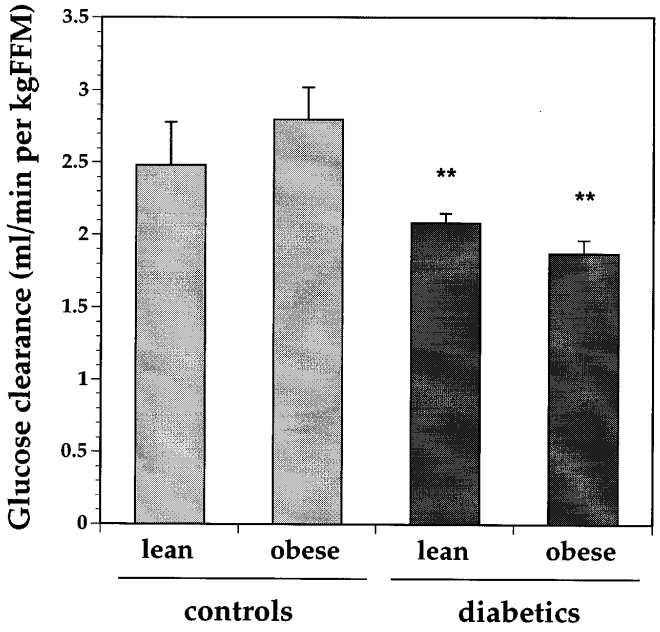

FIG. 2. Rates of plasma glucose clearance in the 4 study groups. Bars are mean values +1 SE. **P < 0.001 vs. nondiabetic subjects.

hyperglycemia should inhibit both pathways (37,38). Therefore, in descriptive terms, it is appropriate to state that in human diabetes, both GNG and glycogenolysis are resistant to insulin and glucose.

With regard to the hormonal control of glucose fluxes, fasting plasma insulin concentrations were raised in association with obesity, whereas plasma glucagon levels were higher in the diabetic subjects and tended to be decreased in obese subjects. Furthermore, plasma glucagon was directly related to EGO and to gluconeogenic flux. Therefore, it can be construed that in the fasting diabetic patient the relative hyperglucagonemia stimulates the gluconeogenic pathway and maintains the activity of glucose-6-phosphatase despite the presence of hyperglycemia, which normally suppresses both (39). In obese subjects, on the other hand, the fasting hyperinsulinemia and the relative hypoglucagonemia restrain glycogenolysis, thereby maintaining euglycemia in the face of enhanced GNG. Although the prehepatic glucagon-to-insulin molar ratio could not be measured in our study, these hormonal data are compatible with the paradigm (40,41) that the liver sinusoidal glucagon-to-insulin ratio is a key regulator of hepatic glucose production and its pathways.

Obviously, this construct can only be a partial description of the EGO regulatory system. Rates of substrate delivery, size of glycogen stores (42), resistance of insulin regulatory enzymes in the gluconeogenic and glycogenolytic pathway, and the influence of other hormones (e.g., leptin [43]) are important determinants of EGO. Thus, liver glycogen depots (35,44) and gluconeogenic precursor delivery are both increased in obesity (34) as well as in poorly controlled diabetes (8). Evidence is available that chronic hyperglycemia upregulates the expression of glucose-6-phosphatase (45) and that suppression of EGO by glucose itself is impaired in type 2 diabetes (39). On the other hand, the ability of insulin to control the activity of glucose-6-phosphatase and to depress the expression of PEP-carboxykinase (46) is com-

promised in diabetes (47,48). Thus, the increase in GNG activity in diabetes is likely to be multifactorial in origin, with both primary defects and adaptive changes. The natural history of the abnormalities of fasting EGO and its components in the emergence of diabetic hyperglycemia remains to be investigated.

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