

# Glucose-Mediated Glucose Disposal in Insulin-Resistant Normoglycemic Relatives of Type 2 Diabetic Patients

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With the aim of investigating glucose-mediated glucose disposal (glucose effectiveness [GE]) in 15 (3 female and 12 male subjects) insulin-resistant normoglycemic relatives of patients with type 2 diabetes (DM2), and 15 age-, sex-, and BMI-matched control subjects without a family history of DM2, we performed 2 studies: 1) a 5-h euglycemic near-normoinsulinemic pancreatic clamp with somatostatin (360 µg/h), insulin (0.25 mU · kg<sup>-1</sup> · min<sup>-1</sup>), glucagon (0.5 ng · kg<sup>-1</sup> · min<sup>-1</sup>), growth hormone (6 ng · kg<sup>-1</sup> · min<sup>-1</sup>), and tritiated glucose infusion and indirect calorimetry; and 2) on a separate day, an identical 5-h clamp but at hyperglycemia (~12 mmol/l) over the last 2 h. Fasting plasma insulin (PI) concentrations were elevated in the relatives compared with control subjects (49 ± 6 vs. 32 ± 5 pmol/l, *P* < 0.04), whereas plasma glucose (PG) was not (5.6 ± 0.1 vs. 5.5 ± 0.1 mmol/l). At the end (i.e., 4.5–5.0 h) of the euglycemic clamp (PG, 6.1 ± 0.4 vs. 5.6 ± 0.1 mmol/l; PI, 78 ± 5 vs. 73 ± 6 pmol/l), peripheral glucose uptake (Rd<sub>euglycemia</sub>) was decreased in the relatives (2.93 ± 0.08 vs. 3.70 ± 0.23 mg · min<sup>-1</sup> · kg<sup>-1</sup> fat free mass [FFM], *P* < 0.005), due to a decreased nonoxidative glucose disposal (0.83 ± 0.21 vs. 1.62 ± 0.19 mg · min<sup>-1</sup> · kg<sup>-1</sup> FFM, *P* < 0.01), but hepatic glucose production (HGP) was increased (1.97 ± 0.19 vs. 1.50 ± 0.13 mg · min<sup>-1</sup> · kg<sup>-1</sup> FFM, *P* < 0.05). At the matched end of the hyperglycemic clamp (PG, 12.7 ± 0.2 vs. 12.6 ± 0.2 mmol/l; PI, 87 ± 5 vs. 78 ± 7 pmol/l), peripheral glucose disposal (Rd<sub>hyperglycemia</sub>) (5.52 ± 0.22 vs. 5.92 ± 0.29 mg · min<sup>-1</sup> · kg<sup>-1</sup> FFM, NS), nonoxidative glucose disposal (2.93 ± 0.18 vs. 2.78 ± 0.25 mg · min<sup>-1</sup> · kg<sup>-1</sup> FFM, NS), and HGP<sub>hyperglycemia</sub> (1.20 ± 0.09 vs. 1.37 ± 0.23 mg · min<sup>-1</sup> · kg<sup>-1</sup> FFM, NS) were all identical. When the effectiveness of glucose itself on glucose uptake and

production [(Rd<sub>hyperglycemia</sub> - Rd<sub>euglycemia</sub>)/ΔPG and (HGP<sub>euglycemia</sub> - HGP<sub>hyperglycemia</sub>)/ΔPG] was calculated, the relatives had a 22% increase in peripheral uptake (0.022 ± 0.002 vs. 0.018 ± 0.002 mg · min<sup>-1</sup> · kg<sup>-1</sup> FFM per mg/dl), due to a significantly increased nonoxidative glucose metabolism and enhanced suppression of HGP (0.0076 ± 0.0021 vs. 0.0011 ± 0.0022 mg · min<sup>-1</sup> · kg<sup>-1</sup> FFM per mg/dl, *P* < 0.05). In conclusion, in insulin-resistant relatives of DM2 patients, whole-body glucose-mediated glucose disposal is increased by GE enhancement of the muscle nonoxidative glucose pathway and by GE enhancement of the suppression of HGP. These mechanisms may represent a compensatory mechanism to the ongoing insulin resistance of these relatives. *Diabetes* 49: 1209–1218, 2000

**N**ormoglycemic relatives of patients with type 2 diabetes (DM2) are at known risk of developing future diabetes (1), and several studies have demonstrated decreased insulin sensitivity (2–6), impaired activation of muscle glycogen synthase by insulin (5,6), and subtle defects of insulin secretion (2,4,7) in such subjects. Most recently, we demonstrated increased glucose-mediated glucose disposal at basal insulin in insulin-resistant normoglycemic first-degree relatives of patients with DM2 (2), using the Bergman minimal model (8). From the latter study, we hypothesized that the increase in glucose sensitivity (*S<sub>g</sub>*) might be a novel compensatory mechanism by which these relatives maintain normal glucose tolerance (2). This scenario was consistent with an earlier large-scale longitudinal follow-up study in normoglycemic relatives of 2 parents with DM2 in whom a decreased *S<sub>g</sub>* and decreased insulin sensitivity (*S<sub>i</sub>*) both predicted an increased risk of future development of diabetes (9). In contrast, Doi et al. (10) noted a decreased *S<sub>g</sub>* with normal *S<sub>i</sub>* and decreased first-phase insulin secretion in normoglycemic relatives of Japanese DM2 patients. However, the estimation of *S<sub>g</sub>* may be influenced by the extent of the acute insulin secretory response, i.e., the lower the first-phase insulin response, the lower the *S<sub>g</sub>* (11–13). Concern has also been expressed as to whether the minimal model technique using unlabeled glucose accurately measures *S<sub>g</sub>* (14,15).

Glucose-mediated glucose disposal is an important factor controlling glucose tolerance (16,17), but which metabolic pathways are involved is uncertain (18–20). Acute hyperglycemia itself is known to enhance peripheral glucose uptake (19–24) associated with enhanced glucose oxidation and glu-

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C-peptide, connecting peptide; DM2, type 2 diabetes; FFA, free fatty acid; FFM, fat-free mass; GE, glucose effectiveness; GE<sub>rd</sub>, peripheral glucose disposal rate; GE<sub>TOTAL</sub>, total glucose disposal rate; GF, glycolytic flux; GH, growth hormone; GINF, glucose infusion rate; GS, glucose storage; HGP, hepatic glucose production; OGTT, oral glucose tolerance test; PG, plasma glucose; PI, plasma insulin; R<sub>a</sub>, rate of appearance; R<sub>d</sub>, rate of disappearance; Rd<sub>euglycemia</sub>, peripheral glucose uptake; Rd<sub>hyperglycemia</sub>, peripheral glucose disposal; SA, specific activity; *S<sub>g</sub>*, glucose sensitivity; *S<sub>i</sub>*, insulin sensitivity.

cose storage (GS) (18–20,24) and to suppress hepatic glucose production (HGP) (19–23,25,26). In chronically hyperglycemic patients with DM2, these mechanisms normalize glucose uptake (27,28) and activation of glycogen synthase (27) in the presence of the ongoing severe insulin resistance and reduced insulin secretion. However, the normoglycemic insulin-resistant relatives in our study had comparable basal and post-oral glucose load plasma glucose concentrations to the control subjects; and yet,  $S_g$  was increased at basal insulinemia (2). This therefore implies an enhanced action of glucose itself on its own disposal in the relatives (2).

In patients with frank DM2, the acute actions of glucose itself on hepatic and peripheral glucose metabolism have been investigated using several different protocols. When a dynamic insulin profile was created similar to a normal nondiabetic oral glucose tolerance test (OGTT) insulin profile, glucose effectiveness (GE) was normal (21). In contrast, when plasma insulin (PI) concentration was maintained constant at the basal level in the DM2 patients (20,22,26), both peripheral and hepatic GE were decreased. However, when GE in DM2 patients was measured at basal insulinemia but with variable glycemic levels, peripheral GE was reduced only when plasma glucose was  $>7$  mmol/l (23), hepatic GE being normal at all glucose levels.

To date, the role of GE in insulin-resistant normoglycemic relatives of DM2 patients, using the glucose clamp technique, has not been reported. The aims of the present study were therefore to 1) evaluate our previous minimal model finding (2) of an increased  $S_g$  at basal insulinemia by using the “gold standard” hyperglycemic-euglycemic somatostatin pancreatic clamp at low physiological insulin concentrations; 2) extend the above findings by combining the clamp with a tritiated glucose infusion and indirect calorimetry to investigate the metabolic pathways (i.e., GS, glycolytic flux (GF), and/or glucose oxidation) involved in the total GE; 3) determine whether the primary effect of GE is in the periphery (i.e., muscle) and/or HGP; and 4) to investigate at which metabolic site the enhanced GE acts as a potential “normalizing” compensatory mechanism in these normoglycemic insulin-resistant relatives.

## RESEARCH DESIGN AND METHODS

**Subjects.** First-degree relatives (children) of patients with DM2 were traced via questioning patients with verified DM2 from the Department of Endocrinology, Odense University Hospital. Fifteen subjects with at least 2 first-degree relatives with DM2 or 1 first-degree relative and at least 2 second-degree relatives with DM2, and 15 normoglycemic control subjects without any family history of DM2, matched according to age, sex, and BMI, were included in the study. All subjects had a normal OGTT and were not on medications known to influence glucose homeostasis (Table 1).

**Protocol.** The subjects were studied twice: after a 10-h overnight fast and after 3 days' intake of  $>150$  g carbohydrate per day.

On day 1, commencing at 8:00 A.M., a hyperglycemic pancreatic clamp was performed. Two polyethylene catheters were inserted: one in an antecubital arm vein for infusion of test substances, and the other in a contralateral heated dorsal hand vein for blood sampling (29). After 30 min (at time 0), a surface-adjusted primed constant [ $^3$ H]glucose (DuPont-New England Nuclear, Boston, MA) infusion was started and continued throughout the study periods (i.e., 0'–60' basal, 60'–240' equilibration, and 240'–360' experimental periods [Fig. 1]). To obtain a common baseline plasma specific activity, the primed-constant tracer infusion was adjusted for individual body surface area as previously described (30). Following the basal period, a pancreatic clamp was commenced by an infusion of somatostatin (360  $\mu$ g/h; Ferring, Kiel, Germany), insulin (0.25 mU  $\cdot$  min $^{-1}$   $\cdot$  kg $^{-1}$  [Actrapid; Novo-Nordisk, Bagsvaerd, Denmark]), glucagon (0.5 ng  $\cdot$  min $^{-1}$   $\cdot$  kg $^{-1}$  [Glucagen; Novo-Nordisk]), and growth hormone (GH) (6 ng  $\cdot$  min $^{-1}$   $\cdot$  kg $^{-1}$  [Norditropin; Novo-Nordisk]) (equilibration period [Fig. 1]). Based on bedside plasma glucose measurements and with a variable infusion of 18% glucose enriched with [ $^3$ H]glucose (HOT-GINF)

TABLE 1  
Clinical characteristics of the study subjects

	Relatives	Control subjects
<i>n</i>	15	15
Age (years)	29.1 $\pm$ 1.5	32.1 $\pm$ 1.4
Sex (F/M)	3/12	3/12
BMI (kg/m $^2$ )	27.2 $\pm$ 0.9	26.6 $\pm$ 1.0
Weight (kg)	86.4 $\pm$ 2.8	85.6 $\pm$ 4.9
FFM (kg)	64.8 $\pm$ 2.5	65.6 $\pm$ 3.5
Waist-to-hip ratio	0.9 $\pm$ 0.02	0.87 $\pm$ 0.02
HbA $_{1c}$ (%)	5.44 $\pm$ 0.10	5.57 $\pm$ 0.08
Fasting plasma glucose (mmol/l)	5.60 $\pm$ 0.09	5.53 $\pm$ 0.06
2-h OGTT plasma glucose (mmol/l)	5.07 $\pm$ 0.34	4.81 $\pm$ 0.34
Fasting plasma insulin (pmol/l)	49.0 $\pm$ 6.1	31.6 $\pm$ 4.8*
Fasting plasma C-peptide (pmol/l)	680 $\pm$ 68	493 $\pm$ 62†
Fasting plasma triglyceride (mmol/l)	1.64 $\pm$ 0.26	1.15 $\pm$ 0.13
Fasting total cholesterol (mmol/l)	5.09 $\pm$ 0.17	4.88 $\pm$ 0.16
Fasting HDL cholesterol (mmol/l)	1.21 $\pm$ 0.08	1.28 $\pm$ 0.10
Fasting LDL cholesterol (mmol/l)	3.15 $\pm$ 0.15	3.08 $\pm$ 0.16

Data are *n* or means  $\pm$  SE. Fasting plasma glucose, insulin, and C-peptide concentrations are the means of 8 determinations from the euglycemic and hyperglycemic study days. \* $P < 0.05$ , relatives vs. control subjects; † $P = 0.05$ .

(60  $\mu$ Ci/500 ml 18% glucose [HOT-GINF-1]) (Fig. 1) (30), plasma glucose (PG) concentrations were clamped at the fasting level. Hyperglycemia was induced at 240 min by a surface-adjusted loading infusion of 18% glucose (enriched with 130  $\mu$ Ci/500 ml 18% [HOT-GINF-2]) for 10 min followed by 120-min clamping of PG concentrations at 12–13 mmol/l (Fig. 1).

On day 2 (performed  $>1$  week after the first study), a euglycemic pancreatic clamp was performed, as described for the hyperglycemic study day, except that euglycemia was maintained during the whole experimental period (HOT-GINF-1) (euglycemic experimental study period 240'–360') by clamping PG at the fasting level (Fig. 1).

Blood samples for measurements of PG concentration were obtained at 10-min intervals for the entire 5-h study. Plasma insulin, tritiated glucose, and tritiated water samples were measured in samples obtained at 20-min intervals for the initial 4.5 h and then each 10 min for the last half-hour. Two basal, 2 equilibration period, and 2 blood samples at the end of the experimental periods were collected for measurements of plasma glucagon, GH, lactate, and free fatty acid (FFA) concentrations. Fasting blood for plasma triglyceride, cholesterol, and HbA $_{1c}$  was also taken.

Indirect calorimetry was performed during the basal (30'–60') and the experimental (330'–360') periods using a computerized flow-through canopy gas analyzer system (Deltatrac; Datex, Helsinki, Finland) as previously described from our laboratory (5,27,31). During the hyperglycemic study day, urine glucose excretion was  $0.11 \pm 0.04$  and  $0.09 \pm 0.04$  mg  $\cdot$  min $^{-1}$   $\cdot$  kg $^{-1}$  fat-free mass (FFM) (NS) in the relatives and control subjects, respectively, which represents  $<2\%$  of the total glucose disappearance only and was therefore not included in the calculations. The protocol was approved by the local ethics committee (protocol number 94/7), informed written consent was obtained from all participants, and all procedures were performed according to the principles of the Helsinki Declaration.

**Assays.** PG was measured at bedside on a Beckman glucose analyzer (Beckman Instruments, Fullerton, CA) by the glucose oxidase method. Plasma insulin and connecting peptide (C-peptide) blood samples were immediately centrifuged at 4°C and stored at  $-20^\circ\text{C}$  for later analysis by 2-site time-resolved immunofluorometric assay (32). Plasma glucagon was assayed after extraction of plasma with 70% ethanol (vol/vol, final concentration), by radioimmunoassay using a COOH-terminal-directed antibody (antibody code

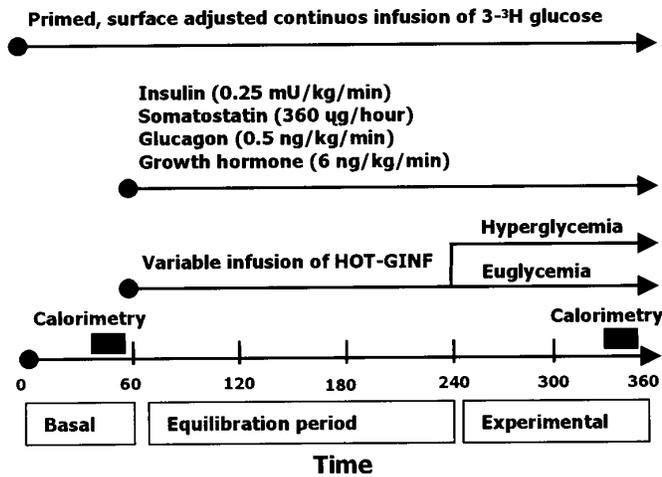


FIG. 1. Design of the study. (The details of the design are explained in the text.)

no. 4305), which measures mainly glucagon of pancreatic origin (33). The sensitivity is  $<1$  pmol/l, and the intra-assay coefficient of variation is  $<6\%$  at 20 pmol/l (33). Tritiated glucose and water ( $[3\text{-}^3\text{H}]\text{glucose}$  and  $^3\text{H}_2\text{O}$ ) concentrations were measured by methods previously described from our laboratory (31). Triglyceride was measured by an enzymatic colorimetric method, as was FFA (Wako Chemicals, Neuss, Germany); plasma lactate by the method of Passonneau (34); and  $\text{HbA}_{1c}$  by high-performance liquid chromatography (normal range 4.1–6.1%).

**Calculations.** Individual total glucose infusion rate (GINF) was calculated as the mean infusion rate during the final 30-min period for both the euglycemic and hyperglycemic study days. Rates of total glucose appearance ( $R_a$ ) and disappearance ( $R_d$ ) were calculated from the measurements of plasma tritiated glucose concentrations under nonsteady state as described from our laboratory (31). HGP was calculated as the difference between  $R_a$  and GINF. Rates of exogenous GF were calculated as described previously (18,31). The calculation is based on the generation of  $^3\text{H}_2\text{O}$  via the glycolytic conversion of the exogenous infusion of  $[3\text{-}^3\text{H}]\text{glucose}$  (35). Rates of GS were calculated as the difference between  $R_d$  and GF at euglycemia and hyperglycemia, and rates of nonoxidative glucose metabolism as the difference between  $R_d$  and glucose oxidation (18,31). Glucose-mediated total glucose disposal rate ( $\text{GE}_{\text{TOTAL}}$ ) was calculated as

$$\text{GE}_{\text{TOTAL}} = \frac{\text{GINF}_{\text{hyperglycemia}} - \text{GINF}_{\text{euglycemia}}}{\text{PG}_{\text{hyperglycemia}} - \text{PG}_{\text{euglycemia}}}$$

peripheral glucose-mediated glucose disposal rate ( $\text{GE}_{\text{Rd}}$ ) as

$$\text{GE}_{\text{Rd}} = \frac{\text{Rd}_{\text{hyperglycemia}} - \text{Rd}_{\text{euglycemia}}}{\text{PG}_{\text{hyperglycemia}} - \text{PG}_{\text{euglycemia}}}$$

and glucose-mediated suppression of HGP ( $\text{GE}_{\text{HGP}}$ ) as (18)

$$\text{GE}_{\text{HGP}} = \frac{\text{HGP}_{\text{euglycemia}} - \text{HGP}_{\text{hyperglycemia}}}{\text{PG}_{\text{hyperglycemia}} - \text{PG}_{\text{euglycemia}}}$$

FFM was calculated as the difference between body weight and total fat mass, the latter being estimated with the bioimpedance method (2). The waist circumference was measured at the level of umbilicus and the hip circumference at the level of the trochanter major.

All data of glucose turnover, GS, and GF are given as milligram per minute per kilogram FFM. GE is expressed as milligram per minute per kilogram FFM per milligram/deciliter.

**Statistical analysis.** The results are presented as means  $\pm$  SE. Differences between the groups or within groups were compared with unpaired or paired  $t$  test. Correlation analyses were performed using Spearman's correlation analysis. Statistical analysis was performed using SPSS for Windows (Version 7.5.1).  $P$  values  $\leq 0.05$  were considered significant. Data are presented as means  $\pm$  SE unless otherwise stated.

## RESULTS

### Fasting plasma insulin, glucose, and lipid concentrations.

When fasting, the relatives had significantly higher PI and

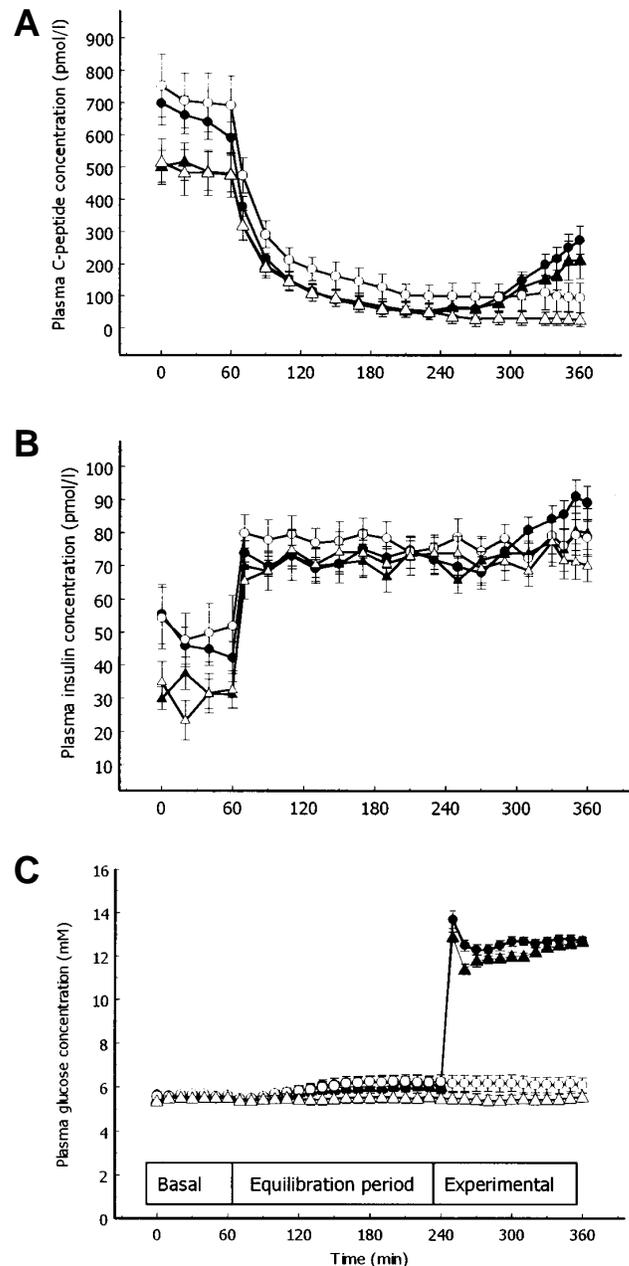


FIG. 2. Time course of plasma C-peptide (A), insulin (B), and glucose (C) concentrations during the euglycemic ( $\circ$ ,  $\Delta$ ) and hyperglycemic ( $\bullet$ ,  $\blacktriangle$ ) study days in 15 relatives of patients with DM2 ( $\circ$ ,  $\bullet$ ) and in 15 control subjects ( $\Delta$ ,  $\blacktriangle$ ).

C-peptide concentrations (Fig. 2A and B and Table 1) compared with those of the age-, sex-, and BMI-matched control subjects, but fasting PG concentrations were similar in both groups (Table 1). No differences existed between the groups with respect to 2-h OGTT plasma glucose concentration, waist-to-hip ratio,  $\text{HbA}_{1c}$  value, or fasting lipid profiles (Table 1).

**Basal and clamp values for plasma insulin, glucose, glucagon, GH, FFA, and lactate concentrations.** With the initiation of the pancreatic clamp, PI concentrations increased and C-peptide concentrations decreased. The expected small breakthrough of endogenous insulin secretion occurred during the final 60 min of the hyperglycemic experimental study

TABLE 2

Plasma glucagon, GH, FFA, and lactate concentrations in relatives and control subjects during the euglycemic and hyperglycemic study days

	Euglycemic		Hyperglycemic	
	Relatives	Control subjects	Relatives	Control subjects
Insulin (pmol/l)				
Basal	50.9 ± 9.0	30.7 ± 5.7*	47.1 ± 5.5	32.6 ± 4.2†
Experimental	78.3 ± 5.1‡	72.6 ± 5.5‡	87.4 ± 4.5‡	78.3 ± 7.3‡§
Glucagon				
Basal	9.70 ± 0.86	7.57 ± 0.51†	9.17 ± 0.68	7.67 ± 0.38*
Experimental	7.13 ± 0.25‡	7.00 ± 0.32	6.00 ± 0.22‡§	6.33 ± 0.25‡
GH (mU/l)				
Basal	0.95 ± 0.24	1.13 ± 0.23	0.45 ± 0.15	0.51 ± 0.15
Experimental	2.93 ± 0.12‡	3.54 ± 0.24†‡	2.93 ± 0.15‡	3.22 ± 0.17‡
FFA (mmol/l)				
Basal	0.38 ± 0.05	0.39 ± 0.04	0.37 ± 0.05	0.36 ± 0.05
Experimental	0.27 ± 0.05	0.16 ± 0.03†‡	0.21 ± 0.05‡§	0.11 ± 0.01*†
Lactate				
Basal	0.77 ± 0.07	0.57 ± 0.04†	0.82 ± 0.08	0.70 ± 0.07
Experimental	0.58 ± 0.06‡	0.53 ± 0.03	0.93 ± 0.06‡§	0.89 ± 0.07‡

Data are means ± SE. \* $P < 0.10$ , relatives vs. control subjects; † $P < 0.05$ ; ‡ $P < 0.01$ , basal vs. experimental period; § $P < 0.05$ , euglycemia vs. hyperglycemia.

period (Table 2, Fig. 2A), although this rise was only significant for the control subjects (Table 2), but there were no differences in the absolute PI concentrations between the 2 groups on either the euglycemic or hyperglycemic study days (330'–360') (Fig. 2B and Table 2).

On the euglycemic study day, the mean experimental period PG concentration (330'–360') changed little compared with the basal concentration in the relatives (6.09 ± 0.37 vs. 5.62 ± 0.10 mmol/l, NS) and control subjects (5.50 ± 0.21 vs. 5.50 ± 0.06 mmol/l, NS) (Fig. 2C). On the hyperglycemic study day, PG concentrations during the experimental period (330'–360') were equally elevated in the relatives and control subjects (12.73 ± 0.21 vs. 12.55 ± 0.18 mmol/l, NS). The coefficients of variation of the clamped PG concentrations were similar for both groups during euglycemia (2.1 ± 0.3 vs. 2.6 ± 0.4%, NS) and hyperglycemia (1.2 ± 0.2 vs. 1.6 ± 0.2%, NS).

Basal plasma glucagon concentrations were increased in the relatives on both the euglycemic and hyperglycemic study days (Table 2). During the somatostatin and hormone replacement pancreatic clamp, plasma glucagon levels in the relatives fell slightly but significantly on both the euglycemic and hyperglycemic study days. This fall was observed only during hyperglycemia for the control subjects (Table 2), consistent with a minor underreplacement of glucagon during the experimental periods. The absolute plasma glucagon concentrations in the relatives and control subjects were similar during the experimental study periods (Table 2). A small but significant increase in the plasma GH concentrations compared with the basal concentrations for both relatives and control subjects was observed during the GH pancreatic clamp infusions (Table 2). Slightly higher GH levels occurred in control subjects during euglycemia (Table 2). These data indicate a consistent small overreplacement of GH.

FFA concentrations declined over time in each group on both the euglycemic and hyperglycemic study days (Table 2), probably reflecting the slight rise in insulin levels during the replacement experimental periods. FFA levels also appeared

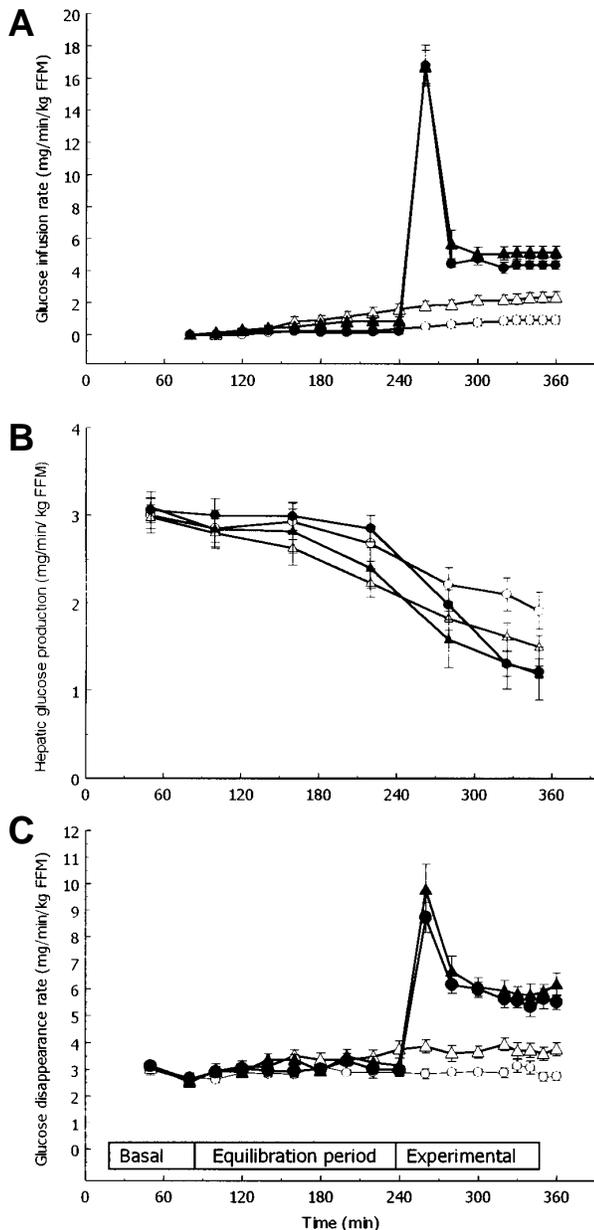
somewhat lower in both groups during the hyperglycemic as compared with the euglycemic experimental period (Table 2). Importantly, the suppression of FFA was less pronounced in the relatives than in the control subjects during both experimental periods (Table 2).

Basal lactate concentrations were significantly decreased in the control subjects on the euglycemic study day; this decrease was not seen on the hyperglycemic study day (Table 2). During the experimental periods, plasma lactate levels decreased or remained unchanged on the euglycemic study day but increased significantly with hyperglycemia in both groups of subjects (Table 2).

**Glucose infusion rates.** On the euglycemic study day, the glucose infusion rate increased over time in both the relatives and control groups (Fig. 3A). Despite the small overall overreplacement of PI and underreplacement of plasma glucagon with the pancreatic clamp, no glucose was infused in 7 of the relatives and 2 of the control subjects. This difference between the groups on the euglycemic study day was reflected in a significantly higher GINF rate in the control group (Table 3). On the hyperglycemic study day, PG concentrations were clamped at comparable levels (Fig. 2C) using the same GINF rates in the relatives and control subjects (Fig. 3A and Table 3).

**Calculated tracer-determined glucose turnover rates.** Specific activities of [3-<sup>3</sup>H]glucose were maintained constant by the HOT-GINF in all study days. The calculated coefficients of variation of the specific activity (SA) values from the euglycemic and hyperglycemic study days were 7.4 ± 1.1 and 8.3 ± 0.7% in relatives and 8.0 ± 0.7 and 12.4 ± 0.9% in the control subjects, respectively.

Basal HGP did not differ between relative and control subjects on either the euglycemic (3.01 ± 0.21 vs. 2.98 ± 0.14 mg · min<sup>-1</sup> · kg<sup>-1</sup> FFM, NS) or hyperglycemic study days (3.06 ± 0.13 vs. 3.09 ± 0.18 mg · min<sup>-1</sup> · kg<sup>-1</sup> FFM, NS) (Fig. 3B). During the pancreatic clamp infusion, HGP fell gradually in both the relatives and control subjects for the euglycemic and hypergly-



**FIG. 3.** Time course of glucose infusion (A), HGP (B), and glucose disappearance (C) rates during the euglycemic (○, △) and hyperglycemic (●, ▲) study days in 15 relatives of patients with DM2 (○, ●) and in 15 control subjects (△, ▲).

cemic study days (Fig. 3B) and was ~25% higher in the relatives than in the control subjects. Interestingly, there was a positive association between HGP during the euglycemic experimental period and basal insulinemia in both the relatives ( $r = 0.76$ ,  $P = 0.001$ ) and the control subjects ( $r = 0.68$ ,  $P = 0.005$ ) and an association between HGP and basal glucagon concentration in the relatives ( $r = 0.49$ ,  $P = 0.06$ ) and the control subjects ( $r = 0.52$ ,  $P < 0.05$ ), reflected by a positive association between the insulin-to-glucagon ratio and HGP in the relatives ( $r = 0.70$ ,  $P < 0.005$ ) and control subjects ( $r = 0.62$ ,  $P < 0.02$ ). During the hyperglycemic study day, HGP was equally suppressed in the relatives and the control subjects (Fig. 3B and Table 3).

Basal tracer-determined rates of total peripheral glucose uptake ( $R_d$ ) were identical between relatives and control subjects on the euglycemic ( $3.02 \pm 0.20$  vs.  $3.03 \pm 0.13$   $\text{mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$  FFM, NS) and hyperglycemic ( $3.13 \pm 0.13$  vs.  $3.14 \pm 0.15$   $\text{mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$  FFM, NS) study days (Fig. 3C). During the euglycemic experimental period,  $R_d$  was unchanged compared with the basal level in the relatives ( $3.02 \pm 0.20$  vs.  $2.93 \pm 0.09$   $\text{mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$  FFM, NS), whereas a significant increase was found for the control subjects ( $3.03 \pm 0.13$  vs.  $3.70 \pm 0.23$   $\text{mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$  FFM,  $P < 0.01$ ) (Fig. 3C). Importantly, the increase in  $R_d$  was significantly greater during euglycemia in the control subjects than in the relatives (Fig. 3C and Table 3). As expected, basal insulin concentrations, a surrogate measure of insulin resistance, correlated inversely with  $R_d$  ( $r = -0.71$ ,  $P < 0.001$ ). With the hyperglycemic study experimental period, a significant increase in the rate of peripheral glucose uptake was noted for each group, with no difference between the relatives and control subjects (Fig. 3C and Table 3). It should be noted that  $R_a$  was greater than  $R_d$  during hyperglycemia in the control subjects ( $6.51 \pm 0.43$  vs.  $5.92 \pm 0.29$   $\text{mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$  FFM,  $P < 0.02$ ), but this was not the case for the relatives ( $5.55 \pm 0.24$  vs.  $5.52 \pm 0.22$   $\text{mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$  FFM, NS). Thus, a steady slow rise in PG concentrations during the hyperglycemic pancreatic clamp occurred in the control subjects (Fig. 2C).

**Glucose and lipid oxidation rates.** Basal glucose oxidation rates did not differ between relatives and control subjects on either the euglycemic ( $2.12 \pm 0.27$  vs.  $1.70 \pm 0.18$   $\text{mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$  FFM, NS) or the hyperglycemic ( $2.13 \pm 0.20$  vs.  $2.27 \pm 0.21$   $\text{mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$  FFM, NS) study day, nor between the 2 study days. Basal lipid oxidation did not differ between relatives and control subjects (euglycemic day:  $1.25 \pm 0.10$  vs.  $1.30 \pm 0.08$   $\text{mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$  FFM, NS; hyperglycemic day:  $1.21 \pm 0.08$  vs.  $1.12 \pm 0.10$   $\text{mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$  FFM, NS).

During the euglycemic experimental period in the control subjects, glucose oxidation rates increased ( $2.09 \pm 0.20$  vs.  $1.70 \pm 0.17$   $\text{mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$  FFM,  $P < 0.04$ ) and lipid oxidation rates decreased ( $1.00 \pm 0.10$  vs.  $1.30 \pm 0.08$   $\text{mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$  FFM) compared with the basal levels. In contrast for the relatives, no differences were noted (glucose oxidation:  $2.10 \pm 0.19$  vs.  $2.12 \pm 0.27$   $\text{mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$  FFM, NS; lipid oxidation:  $1.11 \pm 0.11$  vs.  $1.25 \pm 0.10$   $\text{mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$  FFM, NS). During the hyperglycemic experimental period for both groups, glucose oxidation rates increased (relatives:  $2.59 \pm 0.18$  vs.  $2.13 \pm 0.20$   $\text{mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$  FFM,  $P < 0.02$ ; control subjects:  $3.14 \pm 0.19$  vs.  $2.27 \pm 0.21$   $\text{mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$  FFM,  $P < 0.001$ ) and lipid oxidation rates decreased (relatives:  $0.97 \pm 0.08$  vs.  $1.21 \pm 0.08$   $\text{mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$  FFM,  $P < 0.01$ ; control subjects:  $0.65 \pm 0.07$  vs.  $1.12 \pm 0.10$   $\text{mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$  FFM,  $P < 0.001$ ) compared with the basal levels. No differences existed in glucose or lipid oxidation rates between the groups at euglycemia (Fig. 4 and Table 3). However, during hyperglycemia for the relatives, the changes in glucose oxidation rates and lipid oxidation rates were significantly greater compared with the control subjects (Fig. 4 and Table 3). This result was also reflected by the significant lower respiratory quotient values during hyperglycemia in the relatives compared with the control subjects ( $0.846 \pm 0.008$  vs.  $0.883 \pm 0.008$ ,  $P < 0.01$ ), which was not observed during euglycemia ( $0.827 \pm 0.011$  vs.  $0.831 \pm 0.012$ , NS).

**Glycolytic flux and nonoxidative glucose metabolism.**  $^3\text{H}_2\text{O}$  concentrations during the euglycemic and hyperglycemic study days showed clear linear increases in relatives

TABLE 3

Glucose turnover rates during the experimental periods (330–360 min) of the euglycemic and hyperglycemic study days in relatives and control subjects

		Relatives	Control subjects	P
GINF	Euglycemia	0.92 ± 0.27	2.30 ± 0.35	<0.01
	Hyperglycemia	4.36 ± 0.28*	5.15 ± 0.41*	NS
HGP	Euglycemia	1.97 ± 0.19	1.50 ± 0.13	<0.05
	Hyperglycemia	1.20 ± 0.09†	1.37 ± 0.23	NS
Total peripheral glucose uptake	Euglycemia	2.93 ± 0.09	3.70 ± 0.23	<0.01
	Hyperglycemia	5.52 ± 0.22*	5.92 ± 0.29*	NS
Glucose oxidation	Euglycemia	2.10 ± 0.19	2.09 ± 0.20	NS
	Hyperglycemia	2.59 ± 0.18‡	3.14 ± 0.19*	<0.05
Lipid oxidation	Euglycemia	1.11 ± 0.10	1.00 ± 0.09	NS
	Hyperglycemia	0.97 ± 0.08	0.65 ± 0.07†	<0.005
Exogenous GF	Euglycemia	2.29 ± 0.10	2.42 ± 0.11	NS
	Hyperglycemia	3.04 ± 0.16†	3.12 ± 0.23‡	NS
GS	Euglycemia	0.54 ± 0.16	1.28 ± 0.21	<0.01
	Hyperglycemia	2.48 ± 0.18*	2.81 ± 0.27*	NS
Nonoxidative glucose metabolism	Euglycemia	0.83 ± 0.21	1.62 ± 0.19	<0.01
	Hyperglycemia	2.93 ± 0.18*	2.78 ± 0.25*	NS

Data are means ± SE. All measures are given as mg · min<sup>-1</sup> · kg<sup>-1</sup> FFM. \*P < 0.001; †P < 0.01; ‡P ≤ 0.05, euglycemia vs. hyperglycemia.

and control subjects on both the euglycemic ( $r = 0.89 \pm 0.02$  and  $r = 0.90 \pm 0.02$ ) and hyperglycemic ( $r = 0.92 \pm 0.02$  and  $r = 0.88 \pm 0.02$ ) study days. The calculated exogenous GF rates were similar in the relatives and control subjects during both experimental periods (Table 3), and increased significantly in both groups in response to hyperglycemia (Table 3). A significant correlation between the exogenous GF rate and glucose oxidation during hyperglycemia was found ( $r = 0.58$ ,  $P < 0.001$ ). The GS rate can be calculated as the difference between  $R_d$  and GF, and was significantly reduced in the relatives compared with the control subjects on the euglycemic study day. No difference existed between the relative and control groups during the hyperglycemic study, with both groups rising significantly (Table 3).

Nonoxidative glucose metabolism, calculated as the difference between  $R_d$  and glucose oxidation rate, mirrored the changes observed for GS. As noted above for glucose oxidation, nonoxidative glucose metabolism correlated with the calculated GS rate (from the tritiated water counts) during hyperglycemia ( $r = 0.77$ ,  $P < 0.001$ ).

**Glucose-mediated glucose turnover rates.** On simple inspection of the graph of PG concentration and peripheral glucose uptake ( $R_d$ ) (Fig. 5A), it was noted that the slope of the line relating these parameters differed between the relatives and control subjects. To further evaluate the impact of glucose itself on glucose turnover rates, glucose-mediated total ( $GE_{TOTAL}$ ) and peripheral ( $GE_{Rd}$ ) glucose disposal rates, and the glucose-mediated oxidative and nonoxidative glucose metabolism were calculated.  $GE_{TOTAL}$  was increased by 30% in the relatives compared with the control subjects ( $0.030 \pm 0.003$  vs.  $0.023 \pm 0.003$  mg · min<sup>-1</sup> · kg<sup>-1</sup> FFM per mg/dl,  $P = 0.09$ ) and by 22% for  $GE_{Rd}$  ( $0.022 \pm 0.002$  vs.  $0.018 \pm 0.002$  mg · min<sup>-1</sup> · kg<sup>-1</sup> FFM per mg/dl, NS) (Fig. 5B). When  $GE_{Rd}$  was divided into oxidative and nonoxidative glucose pathways, glucose-mediated nonoxidative glucose metabolism was significantly increased by 50% in the relatives compared with the control subjects ( $0.018 \pm 0.003$  vs.  $0.009 \pm 0.002$  mg · min<sup>-1</sup> · kg<sup>-1</sup> FFM per mg/dl,  $P = 0.03$ ). Glucose-stim-

ulated glucose oxidation rates did not differ between the groups ( $0.0046 \pm 0.0022$  vs.  $0.0084 \pm 0.0019$  mg · min<sup>-1</sup> · kg<sup>-1</sup> FFM per mg/dl, NS). In the relatives, peripheral glucose-mediated glucose uptake was the major contributor to the nonoxidative pathway (nonoxidative  $76 \pm 12$  and oxidative  $24 \pm 12\%$ ), whereas for the control subjects, nonoxidative and oxidative pathways contributed equally to glucose-mediated glucose uptake ( $52 \pm 14$  and  $48 \pm 14\%$ , respectively).

The effect of glucose itself on suppression of HGP was significantly increased in the relatives compared with the control subjects ( $0.0076 \pm 0.0021$  vs.  $0.0011 \pm 0.0022$  mg · min<sup>-1</sup> · kg<sup>-1</sup> FFM per mg/dl,  $P < 0.04$ ) (Fig. 5B). In fact, only in the relatives was a significant effect of glucose itself on HGP documented, whereas in the control subjects, no effect was found (i.e., not significantly different from 0).

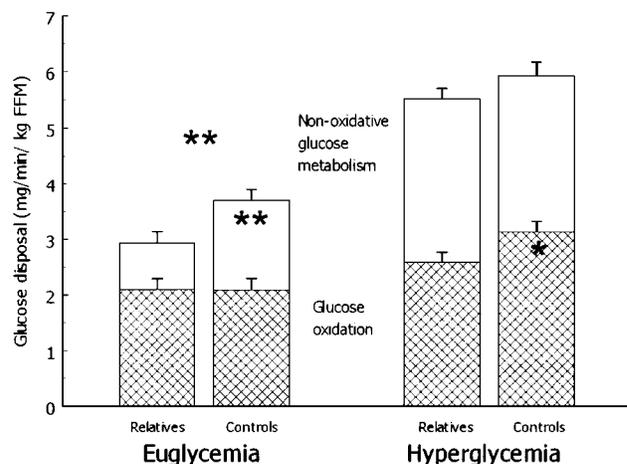
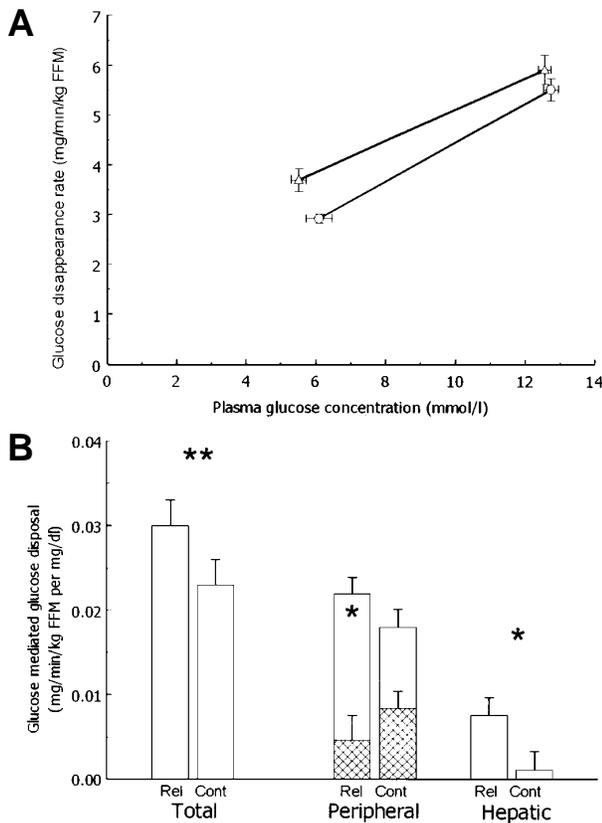


FIG. 4. Peripheral glucose disposal rates in the experimental study periods (330–360 min) during euglycemic and hyperglycemia in relatives of DM2 patients and in control subjects. Bars are divided into oxidative glucose disposal (bottom), and nonoxidative glucose metabolism (calculated as the difference between total peripheral glucose disposal [ $R_d$ ] and glucose oxidation). \*P < 0.05; \*\*P < 0.01 between groups.



**FIG. 5. A:** Clamped PG concentrations and the corresponding peripheral glucose disposal rates in relatives of patients with DM2 (○) and in control subjects (△). **B:** Total glucose-mediated, glucose-mediated peripheral glucose uptake (oxidative [shaded area] and nonoxidative), and glucose-mediated suppression of HGP in relatives of DM2 patients and in control subjects. \* $P < 0.05$ ; \*\* $P < 0.10$  between groups.

**Associations with glucose effectiveness.** To further evaluate the increased GE in the relatives, simple regression analyses of the calculated  $GE_{TOTAL}$  versus different parameters of insulin resistance were examined.  $GE_{TOTAL}$  was associated positively with BMI ( $r = 0.73$ ,  $P < 0.01$ ), fasting insulin ( $r = 0.59$ ,  $P < 0.01$ ), and HGP ( $r = 0.76$ ,  $P < 0.01$ ) at euglycemia in the relatives, and negatively with peripheral  $R_d$  ( $r = -0.61$ ,  $P < 0.05$ ) at euglycemia in the control subjects only.

## DISCUSSION

The present study demonstrates that in insulin-resistant normoglycemic relatives of patients with DM2, glucose-mediated  $GE_{Rd}$  is enhanced and glucose-mediated suppression of HGP ( $GE_{HGP}$ ) is increased in comparison to a group of insulin-sensitive normoglycemic subjects without a family history of DM2. Despite slightly elevated basal PI concentrations, the relatives had reduced peripheral  $R_d$ , which was located in the GS pathway with the euglycemic-hyperinsulinemic clamp technique as previously described (5,6,36). However, at these PI concentrations, suppression of HGP by insulin was impaired in the relatives compared with the control subjects. Together, these data suggest the presence of peripheral and hepatic insulin resistance to the low basal insulin infusion in the relatives. On the other hand, given the positive association noted here between both  $R_d$  and HGP ver-

sus  $GE_{TOTAL}$ , GE appears to play an important compensatory role in overall glucose homeostasis.

In our previous studies in insulin-resistant relatives of DM2 patients using the unmodified minimal model intravenous glucose tolerance test technique, we found a decreased insulin secretion for the degree of insulin resistance and an increased whole-body GE at basal insulin levels (2). There is accumulating evidence that GE may be erroneously estimated in the 1-compartment minimal model (14,15) and may be influenced by the acute insulin secretory response (11–13). It was therefore important to determine whether increased glucose-mediated GE was also present in these relatives using a study design independent of the minimal model assumptions (14) and independent of the influence of the magnitude of the dynamic insulin secretion (11–13,18). Hence the current experimental study design, which used euglycemic and hyperglycemic clamps at low fixed insulin levels performed over 2 days, to estimate the effect of glucose itself on glucose uptake and HGP. With the single-day approach to calculate GE, in which glucose uptake at euglycemia is compared with glucose uptake at subsequent single or multistep hyperglycemia (20,22,23,26), glucose turnover rates change over time, with decreasing glucose disposal and HGP rates (37). This approach would lead to the time-dependent false conclusion that the effect of hyperglycemia on HGP was greater and that the effect on glucose disposal was less. This result was observed in the present study on the euglycemic study day for HGP (Fig. 3B) and would have led us to falsely overestimate the effect of hyperglycemia on HGP. Thus, the current experimental design using a euglycemic control study day was essential to access the real time-independent effect of GE on HGP and  $R_d$ .

The other important aspect of these studies was the level of hormones used during the somatostatin pancreatic clamps. The insulin levels observed during the equilibration and experimental periods of the euglycemic study day were equal but represented ~1.5- and 2-fold increases above the fasting basal insulin levels for the relatives and control subjects, respectively. This small elevation of insulin with the pancreatic clamp was found necessary in preliminary studies to maintain euglycemia in the insulin-resistant relatives (data not shown). With the hyperglycemic study day, similar insulin levels were achieved, although a slight 8–11% breakthrough was seen toward the end of the experimental period in some subjects, the increase being significant for the control subjects only. Glucagon levels indicated minor underreplacement and were suppressed somewhat during hyperglycemia, whereas GH was overreplaced equally in both groups. However, the impact of the small GH excess in the overall replacement hormone admixture on carbohydrate metabolism is probably minor, because FFA levels were suppressed during all equilibration and experimental periods over the 5-h clamps, especially for the normal control subjects. This FFA suppression occurred despite the known lipolytic effect of GH administration (38) seen with higher doses of GH over prolonged time frames (38). Furthermore, it has been shown in sheep that GH does not affect non-insulin-mediated glucose uptake (39).

At the comparable low physiological PI concentration on the euglycemic study day, the GINF necessary to maintain euglycemia in the relatives was significantly decreased compared with that in the control subjects. This may reflect the small 14 pmol/l incremental difference above basal between

PI concentrations for the control subjects ( $\sim 42$  pmol/l) and the relatives ( $\sim 28$  pmol/l). However, the decreased GINF rate, together with the increased fasting PI concentration of the relatives compared with the control subjects and the positive correlation between fasting insulin and  $R_d$  at euglycemia, are consistent with the known peripheral insulin resistance that occurs in such relatives (2-6). A decreased suppression of HGP over time during euglycemia was also noted in the relatives when compared with the control subjects, and HGP during the euglycemic experimental period was significantly increased in the relatives compared with the control subjects. Moreover,  $R_d$  was unchanged over time during the euglycemic study day in the relatives, in contrast to the control subjects, in whom an increase in  $R_d$  was noted over time, despite the same absolute insulin levels in the 2 groups. Thus, for the relatives, the GINF required for maintaining euglycemia was due to a modest decrease in HGP alone, whereas for the control subjects, it was due to a combination of a decreased HGP and an increased  $R_d$ . Also, the suppression of FFA during euglycemia was significantly diminished in the relatives compared with control subjects, despite the similar levels of circulating insulin.

The reduction of  $R_d$  in the relatives at low insulin levels and at euglycemia was due to a decreased peripheral GS (calculated from the tritiated water counts) and a decreased nonoxidative glucose metabolism (calculated from the calorimetry data), which is in agreement with earlier studies using higher clamp insulin levels (5,6,36), in which the defect was linked to decreased insulin stimulation of the muscle enzyme glycogen synthase (5,6). The present study clearly demonstrates that this defect of nonoxidative glucose storage also occurs at low PI concentrations in the relatives. Interestingly, despite normal insulin stimulation of glucose oxidation and GF rates and insulin-mediated inhibition of lipid oxidation in the relatives, FFA concentrations were suppressed less in the relatives than in control subjects.

HGP during the experimental period on the euglycemic study day was significantly higher in the relatives compared with the control subjects despite the comparable insulinemia, with a lesser decrement in HGP from true basal conditions in the relatives. These data are consistent with hepatic insulin resistance in our relatives, a characteristic not uniformly reported for this group (5,6,36,40). Although the small incremental rise of insulin from fasting to the experimental period was lower for the relatives (28 vs. 42 pmol/l), which may be a factor in hepatic insulin sensitivity in the relatives, HGP was similar in relatives and control subjects in the basal situation in which the relatives had significantly increased fasting insulin levels. The latter probably represents a compensatory factor for hepatic insulin resistance in these subjects. Fasting insulin was also positively associated with HGP measured during the low insulin infusion. Although GH was overreplaced in both groups and may have counteracted insulin action on HGP (38,41,42), GH levels were slightly higher in the control subjects in whom hepatic insulin sensitivity was greater. Furthermore, the pancreatic clamp hormone admixture resulted in a small underreplacement of glucagon, particularly for the relatives (due to their higher fasting glucagon concentrations). The lower clamp glucagon should have favored a greater fall in HGP during the experimental euglycemic hyperinsulinemia in the relatives, which was clearly not the case for these subjects. However, fasting glucagon was signi-

ficantly raised in the relatives compared with the control subjects and correlated positively with the HGP during the experimental euglycemic clamp period, but it is not possible to ascertain whether the raised fasting glucagon played a primary role in the hepatic insulin resistance or whether it was secondary to a generalized insulin-resistant state of the relatives. Thus, together these data on HGP during the low basal insulinemic clamp probably reflect a significant resistance of HGP suppression by insulin for the relatives.

During hyperglycemia, HGP was similar in both groups. The finding in the control subjects probably reflects the previously noted effect of time on HGP and indicates that HGP was already maximally suppressed for that insulin level, regardless of the glycemic state of the control subjects.

GINFs necessary for maintaining PG concentrations at the hyperglycemic levels were similar in both groups, as were the calculated peripheral glucose disposal and HGP rates, despite the clear difference observed during the euglycemic studies. Thus, the hyperglycemia per se at the low PI concentration was able to normalize the decreased peripheral glucose disposal and increased HGP rates observed at euglycemia in the relatives. This compensatory GE mechanism in normoglycemic insulin-resistant relatives of type 2 diabetic patients has not been previously reported at such low insulin levels using the glucose clamp technique. Hyperglycemic clamp studies in relatives have been performed by other groups (7,43,44), but insulin secretion was not blocked by simultaneous somatostatin infusion. Therefore, the observed GINFs (7,43,44) reflect the combined effects of both hyperinsulinemia and hyperglycemia (7,43,44) and not the effect of hyperglycemia or hyperinsulinemia alone. This combined effect also applies to the calculation of the  $S_i$  obtained by dividing the GINF at hyperglycemia by the prevailing PI concentration (45). That is, the hyperglycemic  $S_i$  index represents a compensated  $S_i$  index (45) and obscures the true insulin sensitivities.

Hyperglycemia induced significant increases in glucose oxidation and GF rates in both groups, increases consistent with previous studies in normoglycemic humans (20,24,27), in type 2 diabetic patients (27), and in dogs (18). Hyperglycemia also induced an increase in GS and in nonoxidative glucose metabolism in each of the groups. Importantly, the hyperglycemia corrected the reduced nonoxidative glucose metabolism that occurred in the relatives during the euglycemic clamp studies. Thus, hyperglycemia per se does compensate for the decreased insulin-stimulated GS present in the normoglycemic relatives of patients with DM2.

The specific effects of glucose itself (GE) on the various in vivo metabolic pathways were calculated from the incremental differences between the hyperglycemic and euglycemic experimental periods per unit change in PG between the 2 study days (19). Total GE was  $\sim 30\%$  higher in the relatives at the given basal levels compared with that in control subjects, but this difference fell short of statistical significance. If we tested our hypothesis with a 1-tailed  $t$  test, justified by our earlier study (2), total GE was significantly ( $P < 0.05$ ) increased in the relatives. In contrast, glucose-mediated suppression of HGP ( $GE_{HGP}$ ) and glucose-mediated stimulation of nonoxidative glucose metabolism ( $GE_{non-ox}$ ) were significantly (by 2-tailed  $t$  test) increased in the relatives. These data would support our previous speculation that the increased  $S_g$  obtained at the true fasting insulin concentration from the minimal model analysis was a compensatory mechanism for

insulin resistance in these subjects (2). In addition, total GE correlated with several parameters that reflect insulin resistance, i.e., fasting insulin, BMI,  $R_{\text{d}}$ , and HGP. These latter findings suggest that the GE-directed metabolic processes represent compensatory mechanisms for the insulin-resistant state of the relatives. Interestingly, in a recent study, Ader et al. (46) demonstrated that, in old insulin-resistant rats, GE can compensate for their insulin resistance, and that the increased GE was confined to the HGP component of the GE. A brief cautionary note: GE was originally defined as glucose disposal due to hyperglycemia per se at basal (fasting) insulinemia (8), but glucose-mediated glucose disposal is influenced by the prevailing insulinemia of the subjects at the time of testing (11–13,18,47). However, the small increase of insulin at the time of testing GE in this study would have only marginally influenced clamp-derived GE values given the insulin-dose response curve for clamp GE (18,47).

Peripheral glucose-mediated glucose disposal is mainly confined to skeletal muscles (48) in which glucose can be either oxidized or stored as glycogen. In the present study, the relative contributions of glucose-mediated glucose oxidation and storage to  $GE_{\text{TOTAL}}$  in the control subjects were 48 and 52%, respectively, which is a result nearly identical to what we previously demonstrated in dogs at basal insulin (18). On the other hand, in the relatives, the proportions were 24 and 76%, illustrating the preferential partitioning of peripheral glucose-mediated glucose disposal to GS and highlighting the compensatory effect of hyperglycemia per se on the decreased insulin-mediated GS in these subjects (5,6). The mechanism by which glucose per se stimulates GS is unknown. One mechanism could be via a direct allosteric activation of the enzyme glycogen synthase by glucose-6-phosphate in skeletal muscle (27,49,50), a mechanism postulated for the normalization of peripheral glucose uptake at hyperglycemia in patients with DM2 (27,49). Alternatively, the hyperglycemia and the current basal insulinemia could induce an increased responsiveness of the glucose transporter GLUT4 in skeletal muscle to translocation to the plasma membrane (51). Also, GLUT1 may play a role (52–54), since in transgenic mice, overexpression of the GLUT1 glucose transporter resulted in decreased fasting plasma glucose concentrations (52–54). However, from these studies and from our own data, it is not possible to decide whether the increased GE or decreased  $S_{\text{I}}$  is the primary or secondary event in the altered glucose disposal patterns.

In conclusion, normoglycemic relatives of type 2 diabetic patients exhibit multiple metabolic defects at low PI concentrations, including insulin resistance in the GS pathway together with hepatic insulin resistance and impaired suppression of FFA, all of which antedate the development of the impaired glucose tolerance. The insulin resistance is compensated for by the enhanced effect of glucose itself, i.e., GE, on glucose-mediated GS and glucose-mediated suppression of HGP under the current experimental conditions.

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