Increased Glucose Effectiveness in Normoglycemic but Insulin-resistant Relatives of Patients with Non-insulin–dependent Diabetes Mellitus

A Novel Compensatory Mechanism

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Abstract

20 normoglycemic first degree relatives of non-insulin–dependent diabetes mellitus (NIDDM) patients were compared with 20 matched subjects without any family history of diabetes using the intravenous glucose tolerance test with minimal model analysis of glucose disappearance and insulin kinetics. Intravenous glucose tolerance index (Kg) was similar in both groups (1.60±0.14 vs 1.59±0.18, × 10^{-2} min^{-1}, NS). However, insulin sensitivity (Si) was reduced (3.49±0.43 vs 4.80±0.61, × 10^{-4} min^{-1} per mU/liter, $P = 0.05$), whereas glucose effectiveness (Sg) was increased (1.93±0.14 vs 1.52±0.16, × 10^{-2} min^{-1}, $P < 0.05$) in the relatives. Despite insulin resistance neither fasting plasma insulin concentration (7.63±0.48 vs 6.88±0.45, mU/liter, NS) nor first phase insulin responsiveness (Phi1) (3.56±0.53 vs 4.13±0.62, mU/liter min^{-1} per mg/dl, NS) were increased in the relatives. Phi1 was reduced for the degree of insulin resistance in the relatives so that the Phi1 × Si index was lower in the relatives (11.5±2.2 vs 16.7±2.0, × 10^{-4} min^{-2} per mg/dl, $P < 0.05$). Importantly, glucose effectiveness correlated with Kg and with basal glucose oxidation but not with total glucose transporter 4 (GLUT4) content in a basal muscle biopsy. In conclusion we confirm the presence of insulin resistance in first degree relatives of NIDDM patients. However, insulin secretion was altered and reduced for the degree of insulin resistance in the relatives, whereas glucose effectiveness was increased. We hypothesize that increased glucose effectiveness maintains glucose tolerance within normal limits in these "normoinsulinemic" relatives of NIDDM patients. (*J. Clin. Invest.* 1994. 94:1196–1204.)

Key words: insulin resistance • glucose effectiveness • insulin secretion • non-insulin–dependent diabetes mellitus • relatives

Introduction

Non-insulin–dependent diabetes mellitus (NIDDM)[1] has been shown to be associated with reduced insulin sensitivity (1) and with defects in insulin secretion (2). A genetic component is involved in the pathogenesis of the disease, and it has been estimated that about 40% of first degree relatives of patients with NIDDM will develop the disease (3). However, it is still not clear which defect precedes the development of the clinical disease: insulin resistance or deficiency of insulin secretion. Although two studies have shown that relatives of NIDDM patients have alterations in insulin secretion (4, 5), in most studies reduced insulin sensitivity seems to play the most important role in the development of the disease, since insulin resistance has been found in subjects with a family history of NIDDM, and believed to be at risk of developing the disease (6–12). This is supported by a recent study which showed that insulin insensitivity in relatives of NIDDM patients is a marker of future NIDDM (13). In addition several studies have shown that relatives of NIDDM patients are characterized by hyperinsulinemia (6, 8, 9, 11, 14–18). It is believed that the hypersecretion of insulin is compensatory to the insulin resistance, thereby attempting to maintain normal glucose tolerance (19, 20). However, hyperinsulinemia has not been inevitably observed in persons at risk of NIDDM (4, 5, 7, 10, 12), and studies have demonstrated insulin resistance without concomitant hyperinsulinemia (7, 10, 12).

The frequently sampled intravenous glucose tolerance test (FSIGT) with Bergman and co-workers' Minimal Model approach to the analysis of glucose and insulin kinetics (21, 22) is a means by which the interplay between insulin sensitivity and insulin secretion can be simultaneously investigated in an individual (23). Importantly, with this technique it is possible to examine the contribution of the mass action effect of glucose itself (glucose effectiveness) on glucose tolerance (21, 23), a factor ignored in the majority of earlier studies (6, 7, 9–12). Using this approach Martin et al. (13) have shown that reduced insulin sensitivity and reduced glucose effectiveness are both strong predictors of future NIDDM in normal glucose tolerant subjects of two parents with NIDDM.

The aim of this study therefore was to examine the interplay between insulin secretion, insulin sensitivity, and glucose effectiveness in normoglycemic first degree relatives of NIDDM patients, carefully matched for age and anthropometric parame-

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1. *Abbreviations used in this paper:* $AIR_{glucose}$, acute insulin response to glucose; FFM, fat-free mass; FSIGT, frequently sampled intravenous glucose tolerance test; GEZI, glucose effectiveness at zero insulin; GLUT1, glucose transporter 1; GLUT4, glucose transporter 4; HGO, hepatic glucose output; IVGTT, intravenous glucose tolerance test; Kg, intravenous glucose tolerance index; NEFA, nonesterified fatty acids; NIDDM, non-insulin–dependent diabetes mellitus; OGTT, oral glucose tolerance test; PGU: peripheral glucose uptake; Phi1, first phase insulin responsiveness; Phi2, second phase insulin responsiveness; Sg, glucose sensitivity or glucose effectiveness; Si, insulin sensitivity.

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Table I. Clinical Characteristics of the Study Subjects

	Group	
	Relatives	Controls
No. of subjects	20	20
Age, yr	29.4±1.6	29.4±1.7
Sex (F/M)	(8/12)	(8/12)
BMI, kg/m^2	25.1±1.0	25.1±0.9
Weight, kg	76.6±3.3	78.8±4.0
FFM, kg	56.3±3.0	58.1±2.9
WH ratio	0.88±0.02	0.89±0.03
HbA_{1c}, %	6.17±0.13	6.12±0.08
Fasting plasma triglyceride, mmol/liter	1.11±0.13	0.96±0.07
Fasting plasma FFA, mmol/liter	0.40±0.04	0.42±0.03
Fasting plasma glucose, mmol/liter	5.41±0.08	5.16±0.08*
Fasting plasma insulin, mU/liter	7.6±0.5	6.9±0.5

Values are mean±SE. BMI, body mass index; FFM, fat free mass; WH, waist hip; HbA_{1c}, glycated hemoglobin A_{1c}. Fasting plasma glucose and insulin are the means of seven determinations from the OGTT and FSIGT days. * $P < 0.05$.

ters to subjects without any family history of diabetes. For this purpose, we employed the minimal model analysis of the FSIGT, as implemented by the computer program Simulation Analysis and Modeling (SAAM) and conversational (CONSAM) interactive approach, which allows for the simultaneous measurements of the model parameters without the necessity for tolbutamide insulin stimulation during the test procedure (24).

Methods

Subjects. First degree relatives (children) of NIDDM patients were traced by questioning patients with verified NIDDM from the Department of Endocrinology, Odense University Hospital. 20 subjects with at least two first degree relatives with NIDDM or one first degree relative and at least one second degree relative with NIDDM were included in the study. All of the relatives had normal oral glucose tolerance test (OGTT) and were without any medication known to influence glucose homeostasis. The relatives were matched according to age, sex, and body mass index to a group of normoglycemic control subjects without any family history of NIDDM (Table I). Clinical characteristics are given in Table I. None of the subjects in this study had participated in our previously published studies (6, 25).

Protocol. The subjects were studied twice after a 10-h overnight fast. The subjects were requested to have a diet intake of > 150 g carbohydrate per day for 3 d prior to the tests. A 75-g OGTT was performed as the first study in order to ensure normal glucose tolerance at study entry. A polyethylene catheter was inserted into the antecubital vein for blood sampling. Blood samples were obtained at −20, −10, 0, 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 120, 150, and 180 min. All samples were analyzed for plasma glucose and insulin.

On a separate day a frequently sampled intravenous glucose tolerance test (FSIGT) was performed. Two polyethylene catheters were inserted into the antecubital veins. One of the catheters was used for intravenous glucose infusion; the other in the contralateral arm was used for blood sampling. The hand of the blood sampling arm was kept in a heated Plexiglas box during the entire study to obtain arterialization of venous blood. A 300-mg/kg body wt. i.v. glucose load (max 25 g) was given over 1 min as a 25% solution, immediately followed by a 50-ml normal saline flush of the catheter. Blood samples for glucose and insulin assay were collected at the following times: −30, −20, −10, −1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 25, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 140, 160, and 180 min.

Before the intravenous glucose tolerance test (IVGTT), muscle biopsies were performed from m. vastus lateralis using a modified Bergström needle (including suction) under local anesthesia. The biopsies were rapidly frozen in liquid nitrogen and stored at −80°C for later analysis. After the muscle biopsies, but before the intravenous glucose load, indirect calorimetry was performed using a computerized flow-through canopy gas analyzer system (Deltatrac: Datex, Helsinki, Finland). Samples of expired and inspired air were analyzed for oxygen and carbon dioxide concentrations. Oxygen consumption and carbon dioxide production was calculated by the computer and recorded once a minute. After an equilibration period of 10 min, the average gas exchange rate recorded 30 min before the intravenous glucose load was used to calculate rates of glucose and lipid oxidation as previously described (26, 27). The protein oxidation rate was estimated from urinary nitrogen excretion (1 g nitrogen = 6.25 g protein). The protocol was approved by the local ethical committee and informed written consent was obtained from all participants before testing.

Assays. Plasma glucose concentration was measured by the glucose oxidase method on a Glucose Analyzer (Beckman Instruments, Inc., Fullerton, CA). Blood samples for plasma insulin were immediately centrifuged at 4°C and stored at −20°C until analysis. Insulin concentration was measured by a double antibody radioimmunoassay in doublets (Kabi Pharmacia Diagnostics AB, Uppsala, Sweden). Within-assay coefficient of variation was 5.6%; total assay variation was 6.2%; cross-reactivity with proinsulin was 40%. Plasma proinsulin was measured by a two-site, time-resolved immunofluorometric assay (DELFIA) (28). Triglyceride was measured by an enzymatic colorimetric method as was free fatty acid (Wako Chemicals GmbH, Neuss, Germany). HbA_{1c} was estimated by HPLC (normal range 5.4–7.4%).

The frozen muscle biopsies were freeze-dried and dissected free of blood, fat, and connective tissue. The muscle powder (4.9–9.2 mg) was homogenized and a crude membrane fraction was isolated as previously described (29, 30). Samples containing 15 μg of protein were analyzed in duplicate by SDS-PAGE and Western blotting (29) followed by quantification of glucose transporter 4 (GLUT4) by densitometric scanning of immunoreactivity. Protein recovery was identical in the crude membrane fractions from relatives and controls (13.5±0.7 vs. 12.7±0.8 μg protein in crude membranes/mg freeze-dried muscle).

Calculations. The analysis of the FSIGT data was based on Bergman's Minimal Model of glucose disappearance (21) and insulin kinetics (22). Glucose and insulin profiles were analyzed as formerly described using the program, SAAM together with the conversational part of the program (CONSAM) (24, 31).

The minimal model of glucose disappearance yields two parameters; the insulin sensitivity index; Si, and the glucose sensitivity index or glucose effectiveness; Sg (21). The insulin sensitivity index represents the increase in net fractional glucose clearance rate per unit change in plasma insulin concentration following the intravenous glucose load. Glucose effectiveness represents the net fractional glucose clearance rate simply due to the increase in glucose itself in the absence of any increase in insulin concentration above baseline. Importantly, both the insulin sensitivity index and glucose effectiveness involves an inhibition of hepatic glucose output (HGO) and an acceleration of peripheral glucose uptake (PGU) due to insulin and glucose, respectively (23). Furthermore, glucose effectiveness includes a contribution mediated by the preexisting basal insulin status (BIE) and a contribution from hyperglycemia per se in the absence of insulin (GEZI). Thus, Sg = BIE (basal insulin effect) + GEZI (glucose effect at zero insulin). BIE is calculated as insulin sensitivity (Si) multiplied by the measured basal insulin concentration. First phase insulin responsiveness (Phi1) is the amount of insulin (per unit volume) that enters the plasma insulin compartment per unit maximal changes in plasma glucose in response to the intravenous glucose load (22). Second phase insulin responsiveness

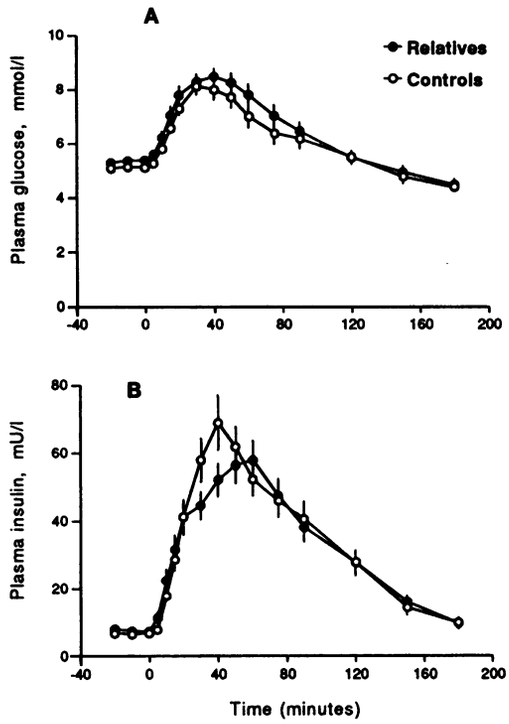

Figure 1. Plasma glucose (*A*) and insulin (*B*) concentrations during oral glucose tolerance test in 20 first degree relatives of NIDDM patients and in 20 control subjects without any family history of diabetes. Means±SE.

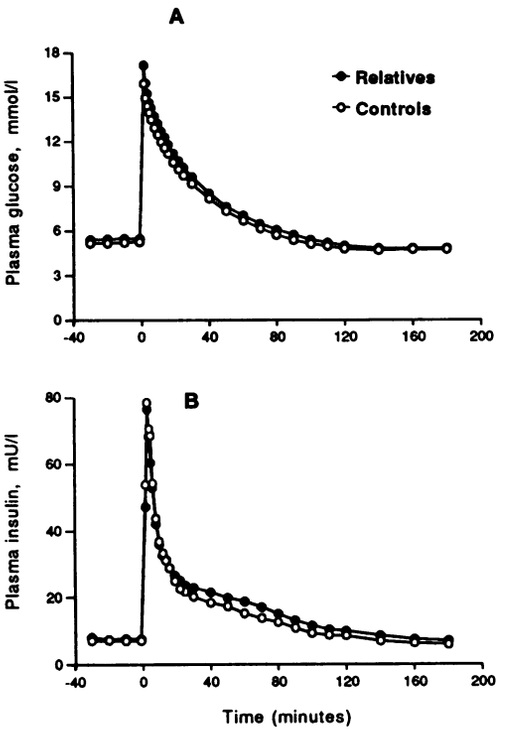

Figure 2. Plasma glucose (*A*) and insulin (*B*) concentrations during intravenous glucose tolerance test in 20 first degree relatives of NIDDM patients and in 20 control subjects without any family history of diabetes.

(Phi2) is the proportionality factor between the rise in glucose concentration above a threshold glucose level and the rise in second phase insulin secretion (22).

The acute insulin response to intravenous glucose ($AIR_{glucose}$) was calculated as the mean of the incremental plasma insulin concentrations from 0–10 min following the intravenous glucose bolus (32). Mean incremental plasma insulin concentration during the OGTT was calculated as the incremental area under the plasma insulin curve (using the trapezoidal method) divided by 180 min. Intravenous glucose tolerance index (absolute Kg) was determined as the least-square slope of the ln of the absolute glucose concentration between 12 and 30 min after the glucose bolus. Moreover, the rate of glucose decay above basal glucose (incremental Kg) was calculated as the least-square slope of the ln of the incremental glucose concentrations between 12 and 30 min. Fat free mass was calculated as the difference between body weight and total fat mass the latter being estimated with the bioimpedance method (33).

Statistical analysis. The results are presented as mean±SE. Differences between the groups were compared by Mann–Whitney rank-sum test. Paired comparisons were performed using the Wilcoxon matched-pairs signed-ranks test. Correlation analyses were performed using Spearman rank sum correlation analysis. P values ≤ 0.05 were considered significant.

Results

The relatives had a small but significantly higher fasting plasma glucose concentration, whereas fasting plasma insulin, triglyceride, and NEFA concentrations were similar to control subjects (Table I). Plasma glucose concentrations following the oral glucose load were similar in both groups (Fig. 1). However, early (0–40 min) plasma insulin concentration following the glucose load appeared to be decreased in the relatives although this did not attain statistical significance; later insulin concentrations were similar in both groups (Fig. 1).

Following the intravenous glucose load, the glucose profiles of the relatives and the control subjects were similar (Fig. 2). The insulin profiles were also similar in the two groups although insulin level appeared to be somewhat higher in relatives of the NIDDM patients during the middle part of the FSIGT (Fig. 2). Fasting and 6, 60, and 120 min plasma proinsulin concentrations were similar in relatives and controls (10.2±1.7 vs 7.0±0.8; 21.3±3.0 vs 16.8±1.6; 21.6±2.4 vs 16.1±1.4; 15.1±2.2 vs 10.8±1.0 pmol/liter NS) as were the ratios between plasma proinsulin and measured plasma insulin (17.8±1.9 vs 13.9±1.1; 6.3±0.6 vs 5.0±0.5; 17.8±1.8 vs 15.5±1.2; 25.4±2.5 vs

Table II. Glucose and Insulin Kinetic Parameters Derived from the FSIGT in the Relatives and the Control Subjects

	Group		
	Relatives	Controls	Units
Kg	1.60±0.14	1.59±0.18	10^{-2} min^{-1}
Sg	1.93±0.14	1.52±0.16*	10^{-2} min^{-1}
GEZI	1.67±0.16	1.22±0.18*	10^{-2} min^{-1}
Phi1	3.56±0.53	4.13±0.62	mU/l min^{-1} per mg/dl
Phi2	10.27±1.05	9.11±1.71	mU/l min^{-2} per mg/dl
Si	3.49±0.43	4.80±0.61‡	10^{-4} min^{-1} per mU/liter
Si × Phi1	11.5±2.2	16.7±2.0*	10^{-4} min^{-2} per mg/dl

Values are mean±SE. Kg, intravenous glucose tolerance index; Sg, glucose effectiveness; GEZI, glucose effectiveness at zero insulin; Phi1, first phase insulin responsiveness; Phi2, second phase insulin responsiveness; Si, insulin sensitivity. * $P < 0.05$; ‡ $P = 0.05$, all others not significant.

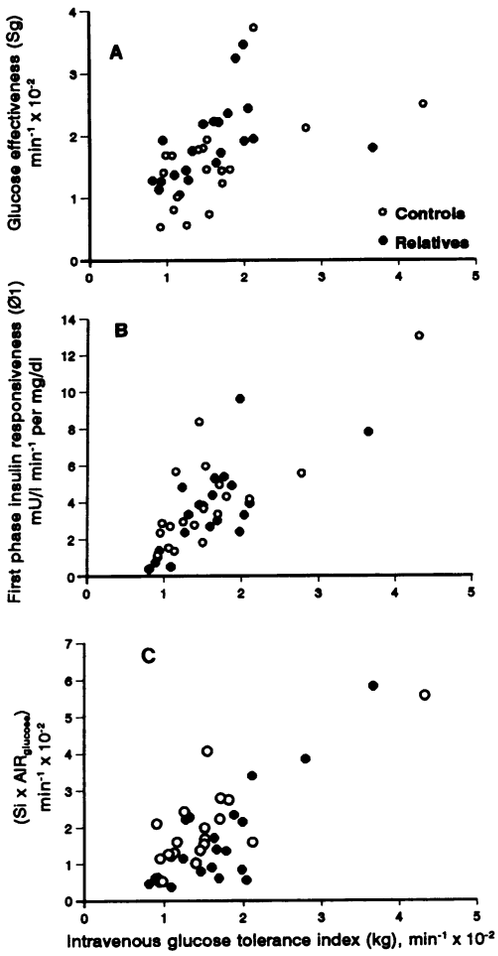

Figure 3. Intravenous glucose tolerance index (Kg) versus glucose effectiveness (Sg) ($R = 0.66$, $P < 0.00001$), first phase insulin responsiveness (Phi1) ($R = 0.71$, $P < 0.00001$) and insulin-mediated glucose disposal (calculated as Si × $AIR_{glucose}$) ($R = 0.87$, $P < 0.00001$).

22.2±1.6%, NS). The intravenous glucose tolerance index, Kg, was similar in both groups (Table II). When these glucose and insulin profiles were subjected to the minimal model analysis, the ability of glucose to promote its own disposal and to inhibit hepatic glucose output at basal insulin, Sg, was significantly increased in the relatives of the NIDDM patients, although there was some overlap between the two groups (Table II). When these data are expressed as the amount of glucose disposed by mass action effect of glucose itself at zero insulin (GEZI), a similar difference between the two groups is observed (Table II). Importantly, there was a significant correlation between Sg and intravenous glucose tolerance index, Kg ($R = 0.66$, $P < 0.00001$) (Fig. 3). Moreover, Sg was inversely related to the fasting plasma glucose concentration in the relatives ($R = -0.45$, $P < 0.05$), but not in the controls ($R = -0.17$, NS).

In the contrast, the insulin secretory parameters Phi1 and Phi2 were similar in both groups (Table II). Interestingly, Phi1 correlated significantly with the 40-min plasma insulin concentration during the OGTT ($R = 0.55$, $P < 0.0003$). In addition there was a positive correlation between Phi1 and Kg ($R = 0.71$, $P < 0.00001$) (Fig. 3). However, as noted above, insulin levels tended to be raised during the middle portion of the IVGTT in the relatives of the NIDDM patients compared to the control

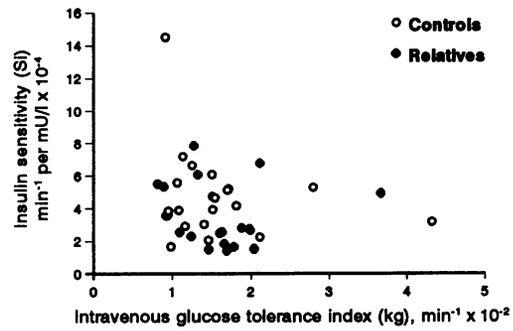

Figure 4. Intravenous glucose tolerance index (Kg) versus insulin sensitivity (Si) ($R = -0.37$, NS).

subjects. As a consequence, we found in the minimal model analysis that the insulin sensitivity (Si) was significantly lower in the relatives (Table II), although overlap did occur between the groups.

No correlation between insulin sensitivity Si and Kg existed ($R = -0.24$, NS) (Fig. 4). However, in order to examine the interplay between insulin sensitivity and insulin secretion, we calculated the insulin-mediated glucose disposal (which includes the ability of insulin to suppress HGO and to accelerate PGU) as the Si × $AIR_{glucose}$ index. A significant positive correlation existed between this parameters and the intravenous glucose tolerance index (Kg) ($R = 0.56$, $P < 0.0002$) (Fig. 3).

If the combined influence of insulin resistance and first phase insulin responsiveness present in the relatives is taken into account by calculation of the glucose disposition index (Si × Phi1) (23, 32, 34), the index is found to be significantly reduced in the relative group (Table II). When these data are represented as the plot between insulin sensitivity (Si) and first phase insulin responsiveness (Phi1), it is evident that only five of the 20 relatives of the NIDDM fell above the line representing the equation Si × Phi1 (equals 16.7 min^{-2} per mg/dl) for the control group (Fig. 5) (sign test $P < 0.05$). The relationship between Si and Phi1 is a hyperbola, i.e., Si × Phi1 = constant (32). We examined whether this relationship between Si and Phi1 existed in the two groups by employing separate regression analysis of Si versus $Phi1^{-1}$ in the two groups. In the control group we found a significant positive correlation ($R = 0.48$, $P < 0.05$), whereas this correlation was absent in the relative group ($R = 0.29$, NS) (Fig. 5). This indicates that the relationship of the Phi1 response to the decreased Si in the relatives is altered and reduced in the group of subjects compared to controls. Moreover, we found a positive correlation between Si and $(\text{fasting insulin})^{-1}$ in the controls ($R = 0.56$, $P = 0.01$) but not in the relatives ($R = 0.26$, NS), thus confirming a hyperbolic relationship between fasting plasma insulin and insulin sensitivity in the controls but not in the relatives. However, the altered insulin secretion may not be the reason for the change to distribution between Si and Phi1 and fasting insulin, respectively, in the relative group, because the insulin secretory parameters Phi1, Phi2, the fasting insulin concentration, and the areas under the OGTT insulin profiles in the presence of the matched dynamic glucose levels were similar in both groups. In order to examine this further we calculated the sum of the insulin-mediated glucose disposal (Si × $AIR_{glucose}$) and glucose-mediated glucose disposal (Sg) (Table III). We found a highly significant correlation between Kg and the sum of insulin and glucose-mediated glucose disposal in the controls ($R = 0.94$, P

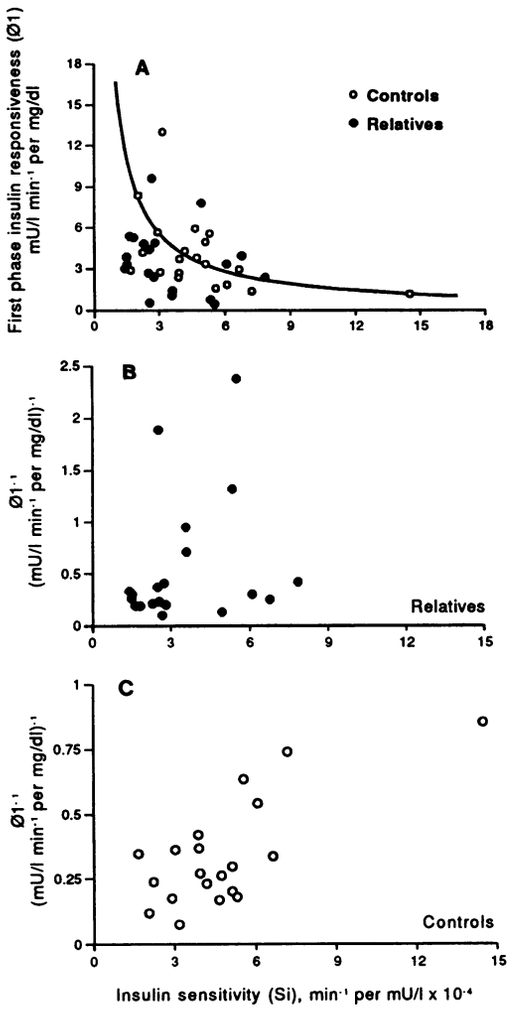

Figure 5. *A*; plot of insulin sensitivity (Si) and first phase insulin responsiveness (Phi1) as estimated from the FSIGT. The line represent the equation Si × Phi1 = 16.7; which is the mean value calculated from the control group. (*B* and *C*); relationship between Si and the inverse of Phi1 in relatives ($R = 0.29$, NS) and controls ($R = 0.48$, $P < 0.05$), respectively.

< 0.00001) as well as in the relatives ($R = 0.81$, $P < 0.00005$) (Fig. 6) indicating that the intravenous glucose tolerance index (Kg) is determined by both insulin-mediated mechanisms and glucose-mediated mechanisms. Interestingly, when the sum of glucose and insulin-mediated glucose disposal was compared to the calculated incremental Kg, which represents the plasma glucose decay rate above basal glucose, no significant differences were observed between these estimates (Table III) in either controls or in relatives. However, in the relatives the increased Sg value contributed for 64±4% of the incremental Kg, whereas in the controls only 42±4% of the incremental Kg was due to Sg (Table III). In addition, when we examined the relative contribution of insulin and glucose-mediated glucose disposal to the glucose restoration rate during the OGTT as proposed by Bergman (23) by using the individual estimated Si and Sg values from the minimal model analysis and the incremental plasma insulin concentration during the OGTT, we found that in the controls 45±4% of the glucose restoration rate was due to insulin-mediated glucose disposal and 55±4% to glucose-mediated glucose disposal (Table III). On the contrary,

Table III. The Contributions of Insulin Sensitivity, Glucose Effectiveness, and Insulin Secretion to Glucose Restoration Rate during the OGTT and the Glucose Decay Rate above Basal during the IVGTT in Controls and Relatives

	Group		
	Relatives	Controls	Units
OGTT			
Mean glucose concentration	6.37±0.20	6.07±0.20	mmol/liter
Mean incremental insulin	25.5±2.4	27.5±2.6	mU/liter
Glucose restoration rate during OGTT			
Due to insulin	0.78±0.07	1.21±0.14‡	10^{-2} min^{-1}
Percent of total	30±3	45±4§	%
Due to glucose (Sg)	1.93±0.07	1.52±0.16*	10^{-2} min^{-1}
Percent of total	70±3	55±4§	%
Total	2.72±0.13	2.74±0.15	10^{-2} min^{-1}
IVGTT			
Incremental Kg	3.40±0.45	3.90±0.46	10^{-2} min^{-1}
Sg	1.93±0.07	1.52±0.16*	10^{-2} min^{-1}
Percent of incremental Kg	64±4	42±4§	%
Si × $AIR_{glucose}$	1.51±0.29	2.11±0.27*	10^{-2} min^{-1}
Sg + (Si × $AIR_{glucose}$)	3.44±0.33	3.63±0.33	10^{-2} min^{-1}

Values are group mean±SE. The glucose restoration rate during the oral glucose tolerance test (OGTT) is due to the combined actions of glucose mediated and insulin mediated glucose disposal, and is calculated as glucose effectiveness (Sg) + insulin sensitivity (Si) × mean incremental insulin during the OGTT (23). The rate of glucose decay above basal (incremental Kg) is due to both the Sg and the enhancement of this rate of glucose disappearance by insulin (Si × $AIR_{glucose}$) (55). Incremental Kg is calculated as the least square slope of the ln of the incremental plasma glucose concentrations (12–30 min) during the intravenous glucose tolerance test (IVGTT) (55), acute insulin response to glucose $AIR_{glucose}$ as the mean insulin concentration above basal (2–10 min) during the IVGTT (32). * $P < 0.05$; ‡ $P < 0.01$, § $P < 0.005$ relatives vs controls, all others NS.

in the relatives only 30±3% of the total glucose disposal was due to insulin-mediated mechanism and 70±3% to glucose-mediated mechanism. However, the total glucose restoration rate was similar in controls and relatives which also is to be expected from the similar plasma glucose concentrations during the OGTTs (Table III).

Please note that the $AIR_{glucose}$ is highly correlated to Phi1

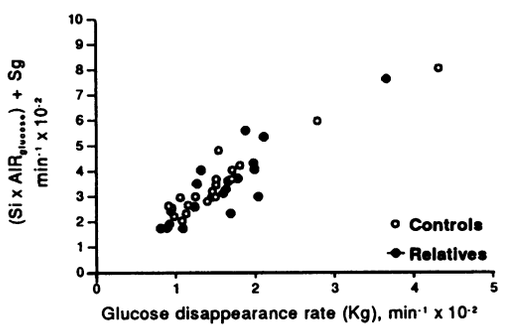

Figure 6. Intravenous glucose tolerance index (Kg) versus the sum of insulin (Si × $AIR_{glucose}$) and glucose (Sg)-mediated glucose disposal ($R = 0.87$, $P < 0.00001$).

Table IV. Basal Glucose and Lipid Oxidation and GLUT4 Content in the Study Subjects

	Group	
	Relatives	Controls
Glucose oxidation (mg/min/kg FFM)	1.94±0.16	1.88±0.19
Lipid oxidation (mg/min/kg FFM)	1.30±0.08	1.21±0.08
GLUT4 content (arbitrary units)	0.51±0.04	0.58±0.03

Values are mean±SE. FFM, fat free mass.

($R = 0.97$, $P < 0.00001$) due to the fact that most of the intersubject variance is found in the plasma insulin concentration ($AIR_{glucose}$: mean 49.1±32.5 (SD), range 8.2–176.0, mU/liter) and that the glucose concentration, for which the insulin concentration has been corrected in calculating Phi1, is very similar between subjects (peak glucose concentration: mean 16.6±2.0, range 13.1–23.1 mmol/liter) compared to the intersubject difference in $AIR_{glucose}$.

GLUT4 content in the crude membrane preparation in the controls and relatives was similar in both groups (Table IV). There was no relationship between Sg, GEZI, SI, or insulin secretory parameters and total GLUT4 content. Finally, basal glucose and lipid oxidation in the relatives and controls were similar in both groups (Table IV). Basal glucose oxidation correlated positively with Sg ($R = 0.44$, $P < 0.005$) and with GEZI ($R = 0.37$, $P < 0.02$) in the overall group indicating that Sg and GEZI are associated with in vivo oxidative glucose metabolism. No correlation between Si and basal glucose oxidation existed ($R = -0.16$, NS).

Discussion

The current results confirm recent studies demonstrating insulin resistance in relatives of NIDDM patients. The defect in insulin action has been localized to the nonoxidative glucose metabolism, i.e., to insulin's ability to enhance glucose storage as glycogen (6, 7, 11, 35) by a reduction in insulin activation of the enzyme glycogen synthase in skeletal muscle (6, 35). Thus, a genetic defect in the enzyme or in the insulin signaling cascade that leads to the activation of the enzyme has been proposed as an inherited trait in NIDDM (1, 6, 35–37).

The novel observation in this study is the increased glucose-mediated glucose disposal (Sg and GEZI) in relatives of NIDDM patients in the presence of reduced insulin sensitivity. Insulin secretion parameters were similar in relatives and controls, although first phase insulin responsiveness was altered and reduced for the given insulin sensitivity in the relatives despite their normal intravenous glucose tolerance index. Moreover, glucose-mediated glucose disposal correlated with basal in vivo glucose oxidation but not with total GLUT4 content. Therefore, increased glucose-mediated glucose disposal may be an important compensatory mechanism by which preservation of normal glucose tolerance can be maintained in these relatives.

We used in our study the frequently sampled intravenous glucose tolerance test together with modeling of glucose and insulin kinetics as a tool for estimating glucose-mediated glucose disposal (Sg) and insulin sensitivity (Si). The method has been validated extensively in the past years. Thus, validation of the estimated glucose effectiveness (Sg) has been carried out using the intravenous glucose tolerance test with endogenous insulin suppressed in normal dogs (38) and in type I diabetics (39). Insulin sensitivity (Si) has been found to be closely correlated to the glucose disposal rate measured by the euglycemic hyperinsulinemic clamp (40–42). Furthermore, the reproducibility of Si and the acute insulin response to glucose has been found to be sufficient (43, 44). One further advantage of the FSIGT is the simultaneous estimation of insulin secretion, insulin sensitivity, and glucose-mediated glucose disposal. However, the insulin sensitivity index involves both the ability of insulin to suppress hepatic glucose output (HGO) and to accelerate peripheral glucose uptake (PGU), and in the same manner the glucose effectiveness involves both glucose-mediated suppression of HGO and acceleration of PGU. Moreover, it must be recognized that both the estimation of Sg (GEZI) and Si can not be separated into the hepatic and peripheral components using unlabeled glucose (45). On the other hand, studies using the euglycemic clamp technique with tracer infusion have not demonstrated hepatic insulin resistance in relatives of NIDDM patients (6, 7, 11). Importantly, however, no studies have directly examined hepatic glucose sensitivity (i.e., the ability of glucose to suppress HGO) in relatives of NIDDM patients.

Only very few studies have examined the mass action of glucose in relatives of NIDDM patients (8, 13, 46) or in patients with frank NIDDM (47–49), although it is known to be an important factor contributing to acute glucose disappearance (23). In NIDDM patients, glucose effectiveness has been found to be reduced in a group of lean Japanese and obese Western NIDDM patients when they were examined by a modified (infusion of exogenous insulin) FSIGT (48, 49). When estimated by the hyperglycemic clamp at zero insulin, NIDDM patients were found to have an increased non-insulin–mediated glucose disposal (47) but the exact physiologic relationship of this latter parameter to Sg or GEZI is not clear. In relatives of NIDDM patients, glucose effectiveness has been found to be normal in one study, although this group of relatives was characterized by pronounced insulin resistance and hyperinsulinemia and was not ideally matched for body weight and composition with their control group (8). On the other hand, in a previous study (13), a low Sg value was found to be a predictor of future NIDDM. However, no control group was included in that study thus making a comparison between relatives of NIDDM patients and persons without a predisposition impossible. Furthermore, it should be noted from that study that reduced insulin sensitivity together with reduced glucose effectiveness seemed to be a stronger predictor of future NIDDM than either reduced insulin sensitivity or reduced glucose effectiveness alone (13). However, our observation of an increased glucose effectiveness is not contrary to the prospective study by Martin et al. (13). First it must be recognized that our study population of relatives represents a mixture of subjects who subsequently will or will not develop diabetes. Second, our observation might suggest that increased glucose effectiveness alone could enhance the glucose tolerance in healthy relatives of NIDDM patients thus preventing them from developing frank diabetes. Thus, in this study the intravenous glucose tolerance index (Kg) correlated positively with first phase insulin responsiveness (as seen in other studies [17, 50, 51]), the calculated insulin-mediated glucose disposal ($Si \times AIR_{glucose}$), and Sg. In particular we found that as much as 64% of the glucose decay rate above basal glucose (incremental Kg) is accounted for by the increased Sg value in the relatives in contrast to only 42% in the controls

thus emphasizing the importance of the increased glucose effectiveness in the relatives. Furthermore, we found, in the relatives and the controls, the same alteration in the proportion between glucose and insulin-mediated glucose disposal during the OGTT as during the FSIGT when employing the method as proposed by Bergman (23). Our data support the conclusion of Kahn et al. (51) concerning the importance of insulin-dependent and insulin-independent glucose uptake for the intravenous glucose tolerance. Therefore, the current data suggest that the raised glucose-mediated glucose disposal (Sg) in our relatives of NIDDM patients represents a novel compensatory mechanism by which the reduced insulin-mediated glucose disposal is compensated thus maintaining normal acute glucose disposal. This conclusion was underscored when we calculated the insulin-mediated glucose disposal as the insulin sensitivity times the mean incremental insulin concentration from 0–10 min following the intravenous glucose bolus and found it to be reduced in the relatives compared with the controls despite the similar intravenous glucose tolerance. Thus, the sum of glucose and insulin-mediated glucose disposal was similar in relatives and controls and furthermore, the sum was similar to the calculated incremental Kg.

It was considered whether the reciprocal change in Si and Sg seen in the relatives could be due to the process of fitting the model to the data. Further calculation of our previously published data in healthy humans by the same fitting process (52, 53) revealed no inverse correlation of Sg with Si (r_s = 0.11 and 0.36, respectively), in conditions where Si was reduced (52) or increased (53) compared to controls. Our previous studies in dogs (54) and in type I diabetic humans (55) provide additional situations where changes in Si due to physiological stimuli are not accompanied by any alterations in Sg. Therefore, these data indicate that reciprocal changes in Sg and Si are not intrinsic to the process of fitting the minimal model to the data. It was also considered whether differences in plasma proinsulin concentrations could explain the slight increase in the middle part of the IVGTT plasma insulin profile of the relatives due to proinsulin cross-reactivity of our insulin assay. However, this seemed not to be the case as proinsulin at all measured time points and the ratios between proinsulin and insulin were similar between relatives and controls in accordance with a previous paper (56). Taking the 60-min samples as an example, the ratio between proinsulin and insulin was 17.8 and 15.5% in the relatives and controls, respectively. Thus, with a proinsulin cross-reactivity of 40% in our insulin assay, 7.1% and 6.2% of the measured plasma insulin is proinsulin. In other words, only a 1% decrease in plasma insulin concentration in the relatives could be expected compared to the controls, if the plasma insulin concentrations had been corrected for proinsulin, which is unlikely to explain the difference observed in the minimal model parameters.

Glucose-mediated glucose disposal in the basal state is primarily observed in non-insulin–sensitive tissue such as the CNS, splanchnic bed, and blood cells. During hyperglycemia the increase in glucose-mediated glucose disposal rate is almost entirely due to an increase of glucose uptake into skeletal muscle (57). Thus, the physiological impact of the higher glucose effectiveness observed in this study could be due to increased skeletal muscle glucose influx. We and others (58–61) have demonstrated that hyperglycemia in NIDDM patients can compensate for the defect in insulin-stimulated glucose disposal. The mechanism behind this compensation is probably via the mass action of glucose thereby increasing the net influx of glucose into the cell. Thus, hyperglycemia in the NIDDM patients was associated with increased free intramuscular free glucose and glucose-6-phosphate concentrations (58) and normalization of the insulin stimulation of the enzyme glycogen synthase (58, 60). As glycogen synthase is stimulated covalently by insulin and allosterically by glucose-6-phosphate, it could be speculated that the increased glucose effectiveness observed in the relatives leads to increased allosteric stimulation of the enzyme thereby compensating for any defect (6, 35) of insulin activation of the enzyme in relatives of NIDDM patients. However, an increased suppression of hepatic glucose output due to glucose itself could be an alternative explanation for the observed increase in Sg in our relatives. The potential importance of the increased Sg in the relatives as a compensatory mechanism is underscored by the small but significantly elevated fasting plasma glucose concentration which correlated inversely with the glucose effectiveness.

Glucose enters the cells through specific glucose transporter molecules. In relatives of NIDDM patients the amount of the insulin-responsive glucose transporter GLUT4 has been found to be normal in one study (62). However, in prediabetic rhesus monkeys it has been shown that the amount of GLUT4 is increased (63). The authors speculated that the increased GLUT4 concentration could be an early response to combat insulin resistance. In our study total GLUT4 content in the crude membrane preparation was similar in both groups. Moreover, no correlation existed between glucose-mediated glucose disposal (Sg) and total GLUT4 content. This was also not likely to be expected since in the basal non-insulin–stimulated state the majority of GLUT4 is located in intracellular storage vesicles and thus does not take part in glucose uptake (64, 65). On the other hand, the GLUT1 glucose transporter is preferentially located to the plasma membrane in the basal state and thus it is believed that GLUT1 mediates the noninsulin–stimulated glucose uptake (64, 65). Recently it was demonstrated that the content of GLUT1 in skeletal muscle of diabetic rats was increased (66) thus leaving the question open as to whether an increased content of GLUT1 could be responsible for the increased Sg in the relatives. Unfortunately, the content of GLUT1 in the human skeletal muscle biopsy can not be evaluated due to inevitable contamination of the biopsy with erythrocytes which express GLUT1 at a very high concentration compared to skeletal muscle cells (67). However, basal glucose oxidation correlated positively with glucose-mediated glucose disposal at basal insulin (Sg) and at zero insulin (GEZI). It could be speculated that the increased glucose-mediated glucose disposal by a mass action effect stimulates glycogen synthase but also by mass action forces glucose into the oxidative glycolytic pathway. Whole body basal glucose oxidation only tended to be increased in the relatives, but since only about 15% of total glucose uptake in the basal state is observed in skeletal muscle (57), the estimation of basal glucose oxidation in skeletal muscle is difficult.

We found an inverse hyperbolic association between insulin sensitivity and fasting insulin concentration and first phase insulin responsiveness in the control subjects. This supports the notion that hypersecretion of insulin may compensate for insulin resistance (19, 20). However, despite this inverse association between insulin concentrations and insulin sensitivity in our subjects, the overall reduced insulin sensitivity observed in the relatives of NIDDM patients was not accompanied by a significantly higher fasting plasma insulin concentration or a higher first or second phase insulin responsiveness. In fact, first phase insulin responsiveness seemed to be reduced in the relatives when their insulin resistance was taken into account. A similar abnormal relationship between insulin sensitivity and acute in-

sulin response to arginine in relatives of NIDDM patients has been observed previously (68) in one study but not in another (8). Interestingly, a low acute insulin response to intravenous glucose recently has been shown to be a predictor of future NIDDM in the Pima Indians, when the degree of obesity and insulin resistance were taken into account (69). Furthermore, the hyperbolic relationship between Si and $AIR_{glucose}$ (32) could not be demonstrated in our relatives whereas this relationship existed in the controls. Recently, Kahn et al. used the IVGTT to quantify the relationship between insulin sensitivity and insulin secretion, and calculated the mean Si $\times$ $AIR_{glucose}$ constant to be 2.24×10^{-2}. In our study the control subjects had a constant very close to this value; 2.11×10^{-2}. In contrast, our relative subjects had a value of 1.51×10^{-2} corresponding approximately to the 25th percentile. However, it can not be concluded unequivocally from this that the lower mean value for the relatives is caused by a defect in beta cell function. Firstly, insulin level in the relatives was similar to controls in the presence of similar glycemia. Secondly, the apparent compensatory increase in glucose-mediated glucose disposal in the relatives may have influenced the setting of the β-cell and therefore it is not possible to conclude whether a primary beta cell defect or an appropriate beta cell function exists in the relatives. However, the early plasma insulin concentration during the OGTT seemed to be reduced in the relatives although this did not attain statistical significance. Since the early plasma insulin concentration during the OGTT correlated with first phase insulin responsiveness from the FSIGT, it is possible that the early OGTT insulin response in the relatives may also be reduced for the degree of insulin resistance as was the Phi1 during the FSIGT.

A defect of insulin secretion as well as insulin resistance is a characteristic feature of the NIDDM syndrome (2). One of the earliest signs of reduced insulin secretion in established NIDDM is loss of first phase insulin secretion to intravenous glucose (2) which has been proposed to be secondary to insulin resistance or mild hyperglycemia (glucose toxicity) (2). However, a primary inherited defect in insulin secretory capacity can not be ruled out. Thus, we have recently demonstrated reduced insulin secretory and insulin resistance in healthy normoglycemic monozygotic twins discordant for NIDDM (70). In light of these speculations, it is interesting to note Bergman's computer simulation of real data (23), where it was shown that simultaneous defects in insulin sensitivity and glucose effectiveness caused a more pronounced deterioration in glucose tolerance than the combined defect in insulin sensitivity and insulin secretion. This supports our hypothesis that the glucose-mediated glucose disposal may be the last defense against the development of glucose intolerance.

In conclusion, we have demonstrated insulin resistance without concomitant hyperinsulinemia in first degree relatives of NIDDM patients. Insulin secretion seemed to be altered if the insulin resistance and fasting glucose were taken into account. The novel observation that glucose-mediated glucose disposal was increased in the relatives and correlated with the intravenous glucose tolerance index Kg and basal glucose oxidation would support the hypothesis that the increased glucose-mediated glucose disposal of these relatives was the physiological mechanism by which normal glucose homeostasis is maintained in these normoinsulinemic insulin-resistant subjects.

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