

Endogenous Glucose Production and Glucose Effectiveness in Type 2 Diabetic Subjects Derived From Stable-Labeled Minimal Model Approach

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Insulin sensitivity, glucose effectiveness, and endogenous glucose production (EGP) during stable-labeled, frequently sampled insulin-modified intravenous glucose tolerance test (FSIGT) were evaluated by a single- and two-compartment minimal model combined with nonparametric deconvolution in eleven nonobese Japanese type 2 diabetic patients. Four patients were treated with sulfonylureas, and the remaining seven with diet therapy alone. None had diabetic retinopathy and microalbuminuria. Their fasting glucose level was 117 ± 7 mg/dl (mean \pm SE), and HbA_{1c} was $6.6 \pm 0.3\%$. Age-, sex-, and BMI-matched subjects with normal glucose tolerance served as control subjects. Plasma insulin response to the stimuli and insulin sensitivity indexes (S_I , S_I^* , and S_I^{2*} were derived from a minimal model and single- and two-compartment-labeled minimal models) were impaired in the type 2 diabetic patients. The combined ability of glucose, per se, to increase its own uptake and suppress EGP (glucose effectiveness [S_G]), which was derived from kinetic analysis of plasma glucose by a minimal model, was significantly lower in the type 2 diabetic patients (0.0132 ± 0.0015 vs. 0.0203 ± 0.0022 ; $P < 0.05$). However, the ability of glucose, per se, to stimulate glucose uptake, assessed as S_G^* and S_G^{2*} from the kinetic analysis of labeled glucose by single- and two-compartment minimal model, was not impaired in those patients. EGP of the type 2 diabetic patients as a whole was suppressed to the level similar to that of the control subjects despite a higher plasma glucose level throughout FSIGT. When EGP in the diabetic subjects was analyzed, considering their recent glycemic control, the initial suppression was blunted in the patients with higher HbA_{1c} levels. In conclusion, glucose mass action to stimulate glucose uptake remains near-normal in the lean Japanese type 2 diabetic patients of this study, whereas ability of glucose to suppress EGP is impaired in the patients with recent hyperglycemia.

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EGP, endogenous glucose production; FSIGT, frequently sampled insulin-modified intravenous glucose tolerance test; OGTT, oral glucose tolerance test; S_G , glucose effectiveness; S_I , insulin sensitivity index.

This blunted suppression of EGP might be one of the conspirators for decreased S_G in subjects with type 2 diabetes. *Diabetes* 48:1054–1060, 1999

The minimal model of glucose disappearance from a frequently sampled intravenous glucose tolerance test (FSIGT) is widely used to assess insulin sensitivity and glucose effectiveness (S_G) in vivo in physiological, pathophysiological, and epidemiological studies (1,2). The model provides simultaneously two metabolic indexes in a single individual, that is, S_G , which is an index of the ability of glucose to increase tissue glucose uptake and suppress endogenous glucose production (EGP), and insulin sensitivity index (S_I). This approach also allows for assessment of insulin secretion normalized to the degree of insulin resistance by the glucose disposition index method (1). S_G plays an important role along with insulin action in the determination of glucose tolerance (3). Reduced glucose mass action has been demonstrated in subjects with type 1 and 2 diabetes (4,5) and impaired glucose tolerance (6) using the minimal model analysis. Moreover, reduced S_G is an important risk factor in the development of type 2 diabetes in a high-risk population (7).

Despite earlier validation studies (8,9), several lines of recent evidence have pointed out that the minimal model systematically overestimates glucose mass action (10–13). One inherent limitation of the original minimal model analysis is that the model is identifiable only using lumped variables. Thus, it is not possible to separate an increase in tissue glucose removal from a decrease in EGP, and both processes are forced to follow the same time characteristics. Additional use of glucose tracers as in the labeled FSIGT was aimed to separate input from the output response of the system. This approach gave peripheral tissue-specific glucose mass action (S_G^*) and insulin sensitivity index (S_I^*), analyzing only disappearance of tracers by the minimal model analysis (14,15). However, the model yielded a physiologically implausible time course of EGP, most probably caused by the monocompartmental description of the glucose system (16). Subsequently, Caumo and Cobelli (16) have published an improved model, featuring two glucose compartments. The newly developed model allows the reconstruction by deconvolution of physiologically plausible EGP (16), and the estimation of a new set of indexes (S_G^{2*} and S_I^{2*}) for glucose uptake (13).

In this context, the present study was designed to reevaluate S_G and S_I and reconstruct a profile of EGP in type 2 diabetic

subjects. Age-, sex-, and BMI-matched subjects with normal glucose tolerance served as control subjects. This is the first clinical study on well-characterized patients with type 2 diabetes using the stable-labeled two-compartment minimal model.

RESEARCH DESIGN AND METHODS

Subjects. A total of 11 nonobese (BMI <25) type 2 diabetic patients and 8 age-, sex-, and BMI-matched healthy subjects were enrolled in the study. The characteristics of the subjects are shown in Table 1. Percent body fat measured by a bioelectrical impedance method (TBF-305, Tanita, Tokyo, Japan) was comparable between these two groups. The control subjects had no family history of diabetes, and a routine health examination revealed no abnormal findings. All the diabetic subjects showed typical gradual onset of type 2 diabetes, having past maximal BMI ranging from 22.7 to 29.4 kg/m² (24.8 ± 0.8, mean ± SE). Family history of type 2 diabetes was recorded in 9 of 11 subjects. Seven subjects were treated with diet therapy alone and four with sulfonylureas, and HbA_{1c} measured by high-performance liquid chromatography method (HA-8131, Kyoto Daiichi-Kagaku, Kyoto, Japan) varied from 5.3 to 8.8% (normal range: 4.3–5.8%) at the time of the study. Diabetic retinopathy and microalbuminuria (>30 mg/day) were not present in all the diabetic subjects, but one subject had symptomatic peripheral neuropathy. Anti-GAD₆₅ antibody (measured with a radioimmunoassay kit; RIP Anti-GAD Hoechst, Tokyo, Japan) (17) was negative in all the diabetic patients. All the subjects underwent both 75-g oral glucose tolerance test (OGTT) and FSIGT. The tests were performed at least 7 days apart, and on the previous day to the 75-g OGTT and FSIGT, they were provided with a supper containing >140 g carbohydrate, >30 g fat, and >33 g protein. The protocol was approved by the local ethical committee in Jichi Medical School, and all the subjects gave their informed consent. **The 75-g OGTT and FSIGT.** The subjects were given 75 g of glucose orally after an overnight fast. Blood samples were obtained before and 30, 60, 90, and 120 min after the load and subjected to measurements of plasma glucose and insulin.

On the day of FSIGT, after an overnight fast, the subjects were admitted to Division of Endocrinology and Metabolism in Jichi Medical School Hospital. Insulin-modified FSIGT was performed in a reclining position as described previously (5). In brief, baseline samples for glucose and insulin were obtained before the injection of 0.3 g/kg glucose isotopically labeled with [6,6-²H₂]glucose (Aldrich, Milwaukee, WI). Chemical purity was verified by specific enzymatic analysis with glucose oxidase. Sterility of the solution was verified by bacteriological analysis, and the material was shown to be pyrogen-free. The final concentration of [6,6-²H₂]glucose in the dose was ~20%. The glucose solution was administered to the antecubital vein, and an additional infusion of regular insulin (Humulin, Shionogi, Osaka, Japan) was performed (20 mU/kg) from 20 to 25 min after the glucose bolus. Blood samples were frequently obtained up to 180 min.

Biochemical and stable isotope tracer analysis. Plasma glucose concentration was determined by the glucose oxidase method by an automatic analyzer (Hitachi 7250, Hitachi, Japan). Plasma immunoreactive insulin was measured in duplicate with a radioimmunoassay kit (Shionogi, Osaka, Japan).

Deuterated glucose was analyzed as a penta-acetate derivative using the method by Wolfe (18). Samples were analyzed on a quadrupole gas chromatography mass spectrometry instrument (GCMS-QP1100EX, Shimadzu, Kyoto, Japan) operated in the electron impact mode by selective-ion monitoring of *m/z* 200 and 202. Oven temperature was 180°C with a rate of temperature rise at 10°C/min until 250°C with a 25-m HR-1 capillary column (Shinwa Chemical Industries, Kyoto, Japan). From tracer to tracee mass ratio of the sample, tracer concentrations were calculated (19). The measurement error associated with the

TABLE 1
Clinical characteristics of control subjects and type 2 diabetic patients

	Control subjects	Type 2 diabetic patients
<i>n</i>	8	11
Sex (M/F)	3/5	4/7
Age (years)	41.1 ± 2.7	45.4 ± 3.2
BMI (kg/m ²)	21.4 ± 0.5	20.9 ± 0.8
Body fat (%)	24.6 ± 1.5	23.8 ± 2.1
HbA _{1c} (%)		6.6 ± 0.3
Duration of type 2 diabetes (years)		5.1 ± 1.8

Data are means ± SE.

labeled glucose measurement was assumed to be independent, white and Gaussian, with zero mean and a coefficient of variation of 3.0%.

K_c, insulin area, and single-compartment minimal model. The glucose disappearance constant (*K_c*) and the area under the curve between 0 and 10 min (or 20 min) after the glucose load were calculated using previously described method (5). Kinetic analysis of plasma glucose concentration was achieved by applying the minimal model (1).

Impulse response described by two-compartment minimal model. The two-compartment minimal model, proposed by Caumo and Cobelli (16), is described in its uniquely identifiable parameterization by the following equations (13):

$$\begin{aligned} dq_1/dt &= -[k_p + R_{d0}/Q_1(t) + k_{21}]q_1(t) + k_{12}q_2(t) - q_1(0) = d \\ dq_2/dt &= k_{21}q_1(t) - [k_{02} + x(t) + k_{12}]q_2(t) - q_2(0) = 0 \\ dx/dt &= -k_b x(t) + k_a [I(t) - Ib] - x(0) = 0 \\ g(t) &= q_1(t)/V_1 \\ K_p &= 3k_{21}k_{02}/(k_{02} + k_{12}) - R_{d0}/V_1/G_b \end{aligned}$$

where *q*₁ and *q*₂ denote labeled glucose masses (milligram per kilogram) in the first (accessible pool) and second compartment, respectively; *x*(*t*) is insulin action (min⁻¹), *I*(*t*), and *Ib* are plasma insulin and basal insulin (microunits per milliliter), respectively; *q*₁(*t*) is cold glucose mass in the accessible pool (milligram per kilogram), *g*(*t*) is plasma-stable glucose concentration (milligram per deciliter), *d* is the labeled glucose dose (milligram per kilogram), *V*₁ is the volume of the first pool (deciliter per kilogram), and *k*₂₁ (min⁻¹), *k*₁₂ (min⁻¹), *k*₀₂ (min⁻¹), *k*_b (min⁻¹), and *k*_a (ml · μU⁻¹ · min⁻²) are parameters describing glucose kinetics and insulin action. *K*_p represents the proportional term of glucose removal from the accessible pool. *S*₁^{2*} and *S*_G^{2*} were defined as follows:

$$\begin{aligned} S_1^{2*} &= (k_a/k_b) k_{21} k_{12} / (k_{02} + k_{12})^2 \\ S_G^{2*} &= k_p + k_{21} k_{02} / (k_{02} + k_{12}) \end{aligned}$$

Thus, units of *S*₁^{2*} (microunits per milliliter per minute) and *S*_G^{2*} (min⁻¹) are expressed in the same fractional indexes as those of *S*₁ and *S*_G of the single-compartment minimal model to allow the comparison among these indexes. The model parameters were estimated using the whole data (3–180 min), and weights were chosen equal to the inverse of the measurement error variance as suggested previously (13,16).

EGP estimated by nonparametric deconvolution. EGP was estimated by nonparametric deconvolution (16,20,21). Briefly, endogenous glucose production (*p*), endogenous glucose concentration (*G_e*), and the impulse response of the system (*h*) given by two-compartment minimal model are related by the integral equation.

$$G_e(t) = \int_0^t h(t, \tau) p(\tau) d\tau + p_b \int_{-\infty}^0 h(t, \tau) d\tau$$

where *p*_b is basal endogenous glucose production. Nonparametric deconvolution was carried out with regularization parameter turned in each individual between 0.1 and 1,000 (×10³). It was 32.1 ± 4.6 (×10³) and 25.3 ± 5.6 (×10³) for the control and diabetic subjects, respectively. Reconvolution resulted in endogenous glucose concentrations similar to the measured values in all the subjects (data not shown).

Programs were written in Pascal (Borland International, Scotts Valley, CA) on Power Macintosh 7300/180 (Apple Computer, Cupertino, CA). In particular, source programs (rk4, mrqmin, gaussj, ludcmp, and lubksb) supplied by Press et al. (22) have been adapted to the particular situation of the minimal model and nonparametric deconvolution.

Statistics. Data were expressed as means ± SE. To evaluate the differences between the patients with type 2 diabetes and the control subjects, data were analyzed by Student's *t* test. A *P* value <0.05 was considered significant.

RESULTS

Glucose intolerance and impaired insulin secretion were observed during the 75-g OGTT and FSIGT in the diabetic subjects (Figs. 1 and 2A and B). Plasma glucose concentrations during 75-g OGTT were significantly higher in the diabetic subjects than those of the control subjects (Fig. 1A). The results of 75-g OGTT in the control subjects were neither impaired glucose tolerance nor diabetes defined according to the World Health Organization criteria. Plasma insulin response to the glucose load was blunted in the diabetic subjects, and the levels of insulin were significantly lower at

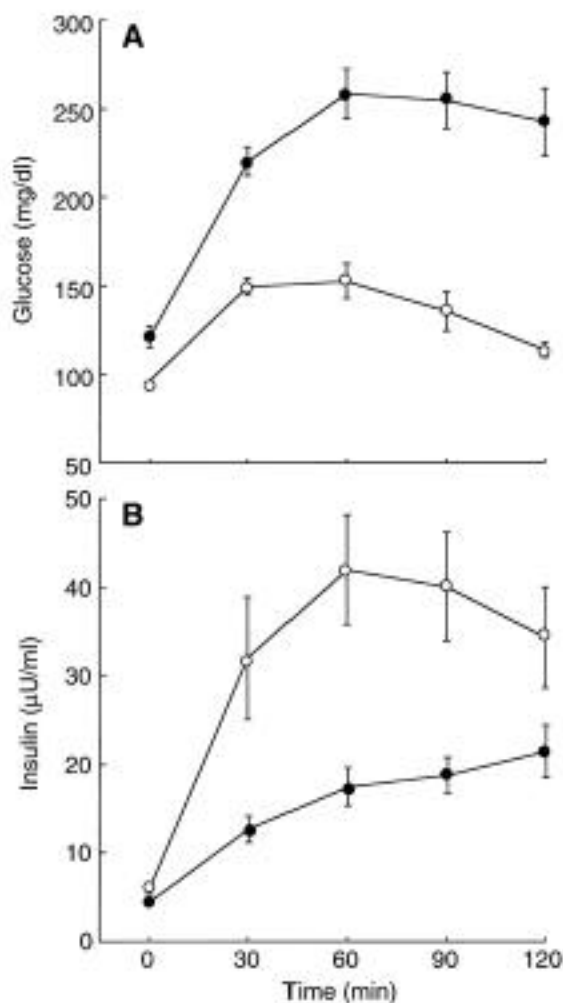


FIG. 1. Plasma glucose (A) and insulin (B) concentrations during 75-g OGTT for normal subjects ($n = 8$) (○) and type 2 diabetic patients ($n = 11$) (●). Values are means \pm SE.

30, 60, 90, and 120 min during 75-g OGTT (Fig. 1B). K_G values of FSIGT were significantly lower in the diabetic subjects (Table 2). Also, an integrated area of insulin above the basal level during the first 10 min of FSIGT, as an index of first-phase insulin secretion, was decreased in the patients with type 2 diabetes (Fig. 2B, Table 2).

S_G and S_I were significantly lower in the patients with type 2 diabetes than those in the control subjects (Table 2). Use of the labeled glucose concentration allowed construction of the exogenous and endogenous component of total plasma glucose concentration in a structure-independent fashion (Fig. 2C and D). Kinetics of exogenous glucose analyzed by a single- and two-compartment minimal model resulted in an impaired insulin sensitivity (S_I^* and S_I^{2*}) in the patients with type 2 diabetes, whereas glucose effectiveness (S_G^* and S_G^{2*}) was not significantly decreased (Table 2).

EGP estimated by nonparametric deconvolution revealed slightly but not significantly ($P = 0.0724$) elevated basal EGP in the type 2 diabetic patients (Table 2). EGP of the type 2 diabetic and control subjects was suppressed in a similar manner, reaching its nadir at ~ 30 min (Fig. 3A). In the control subjects, EGP rapidly increased and recovered to the basal level by 120 min. In contrast, the increase in EGP was delayed, and

TABLE 2
Metabolic parameters of control subjects and type 2 diabetic patients

	Control subjects	Type 2 diabetic patients
n	8	11
Basal glucose (mg/dl)	91 ± 8	$117 \pm 7^\dagger$
Basal insulin ($\mu\text{U/ml}$)	5.1 ± 0.3	4.5 ± 0.4
K_G ($\% \text{ min}^{-1}$)	1.94 ± 0.12	$1.15 \pm 0.10^\dagger$
Insulin area [($\mu\text{U/ml}$) 10 min]	165 ± 51	$26 \pm 10^\dagger$
Insulin area [($\mu\text{U/ml}$) 20 min]	275 ± 68	$54 \pm 14^\dagger$
Minimal model analysis		
S_G (min^{-1})	0.0203 ± 0.0022	$0.0132 \pm 0.0015^*$
$S_I \times 10^4$ [($\mu\text{U/ml}$) $^{-1} \cdot \text{min}^{-1}$]	11.8 ± 2.6	$6.7 \pm 0.8^*$
Labeled minimal model analysis		
S_G^* (min^{-1})	0.0079 ± 0.0006	0.0069 ± 0.0004
$S_I^* \times 10^4$ [($\mu\text{U/ml}$) $^{-1} \cdot \text{min}^{-1}$]	11.0 ± 1.9	$6.2 \pm 0.7^*$
Two-compartment labeled minimal model analysis		
S_G^{2*} (min^{-1})	0.0055 ± 0.0016	0.0070 ± 0.0012
$S_I^{2*} \times 10^4$ [($\mu\text{U/ml}$) $^{-1} \cdot \text{min}^{-1}$]	24.8 ± 2.8	$16.3 \pm 2.5^*$
Basal EGP ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	1.43 ± 0.17	1.77 ± 0.09

Data are means \pm SE. The estimate precisions were $15.4 \pm 1.9\%$ for S_G , $8.7 \pm 1.3\%$ for S_I , $5.6 \pm 0.4\%$ for S_G^* , $8.6 \pm 1.0\%$ for S_I^* , $36.0 \pm 10.6\%$ for S_G^{2*} , and $11.7 \pm 0.9\%$ for S_I^{2*} , respectively. * $P < 0.05$; $^\dagger P < 0.01$, respectively.

EGP was still lower than the basal level at 180 min in the diabetic subjects. When the diabetic patients were divided into two subgroups according to HbA_{1c} level, the initial suppression of EGP was blunted in the patients with poorer glycemic control (HbA_{1c} $> 6.6\%$, $n = 5$), in comparison to the subgroup with better glycemic control (HbA_{1c} $< 6.6\%$, $n = 6$) (Fig. 3B). The differences of EGP between the basal level and the level at 20 min (when an additional insulin infusion was started) were 0.67 ± 0.32 in the former and $1.51 \pm 0.15 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in the latter group ($P = 0.0345$), respectively.

There were significant correlations among S_I , S_I^* , and S_I^{2*} in all the studied subjects: $r = 0.941$, $P < 0.0001$ between S_I and S_I^* ; $r = 0.558$, $P = 0.0131$ between S_I and S_I^{2*} ; and $r = 0.698$, $P = 0.0009$ between S_I^* and S_I^{2*} , respectively. Similarly, in all the subjects, only a correlation between S_G^* and S_G^{2*} was significant among S_G , S_G^* , and S_G^{2*} : $r = 0.301$ between S_G and S_G^* , $r = 0.004$ between S_G and S_G^{2*} , and $r = 0.554$, $P = 0.0138$ between S_G^* and S_G^{2*} , respectively. When the distribution volumes are considered and the labeled indexes are corrected using the distribution volumes (V_1 : $1.25 \pm 0.07 \text{ dl/kg}$ in the two-compartment model, and V : $1.92 \pm 0.09 \text{ dl/kg}$ in the single-compartment model), the correlations remained unchanged (data not shown).

DISCUSSION

The tissue glucose uptake occurs via the facilitated transport system, and the major factors that regulate glucose uptake are the prevailing plasma glucose and insulin concentration. In addition to the well-established presence of insulin resis-

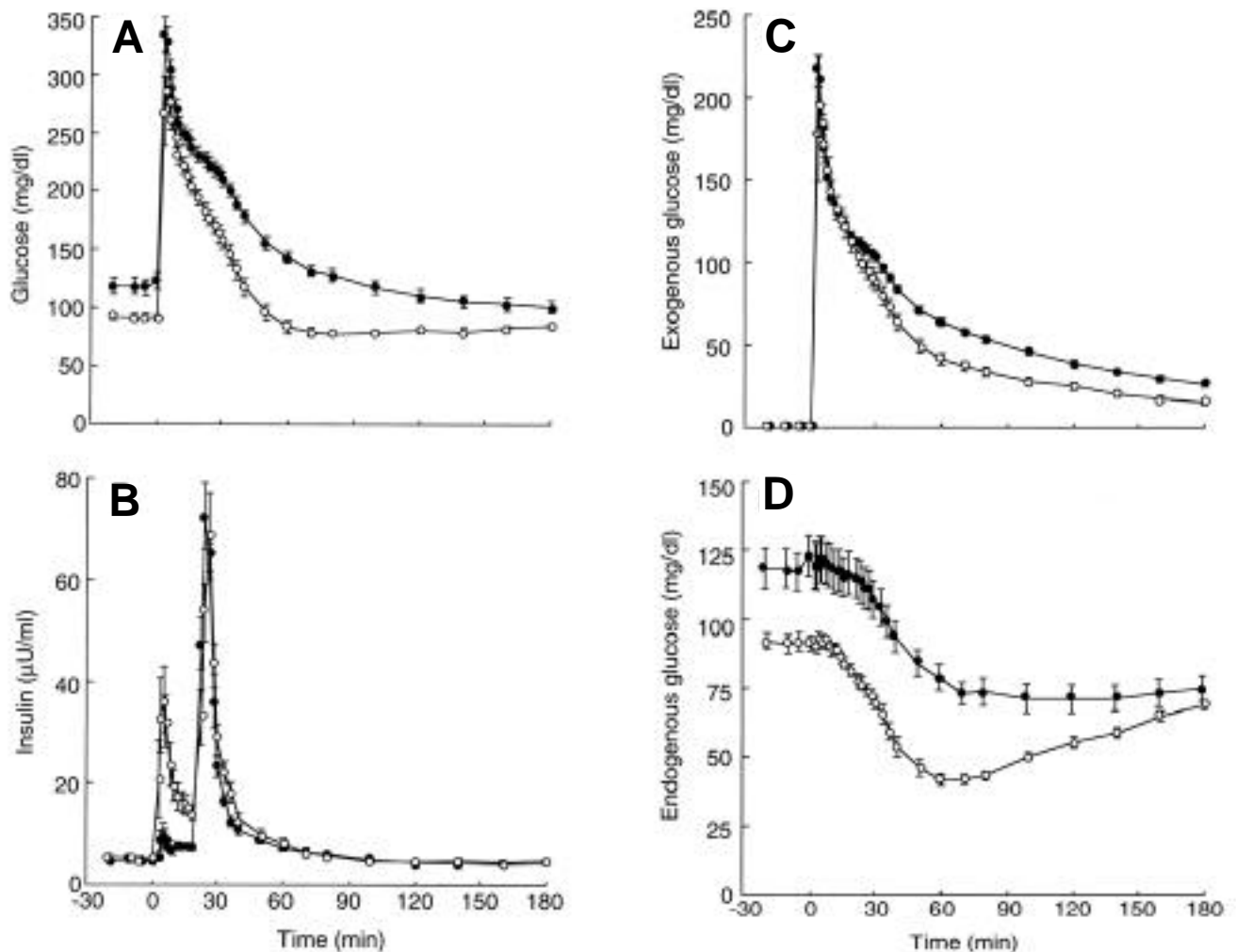


FIG. 2. Plasma glucose (A), insulin (B), exogenous glucose (C), and endogenous glucose (D) concentrations during FSIGT for normal subjects ($n = 8$) (○) and type 2 diabetic patients ($n = 11$) (●). Values are means \pm SE.

tance, also confirmed in the present study, resistance to the effect of hyperglycemia, per se, on its normalization manifests itself in states of glucose intolerance. Diminished glucose mass action, observed as reduced S_G by the minimal model approach, is a common finding in type 1 and 2 diabetic subjects and in subjects with impaired glucose tolerance (4–6). Consistent with the previous studies, S_G in the type 2 diabetic patients in the present study was significantly decreased, compared with that in the control subjects. One of the major findings in this study is a near-normal suppression followed by a delayed recovery of EGP during FSIGT in the patients with type 2 diabetes as a whole. The other is that the stimulatory effect of hyperglycemia on peripheral tissue glucose disposal assessed by S_G^* and S_G^{2*} from the labeled minimal model analysis was not significantly impaired in the type 2 diabetic patients.

Basal EGP values in the control subjects of this study were slightly lower than those of the control subjects estimated by the similar analytical approach (16,21) or were recently quantified by radiolabeled glucose infusion (23,24). The slight difference in EGP may be related to the differences in experimental conditions and/or examined races. In the latter studies, EGP values showed significant correlations with fasting glucose levels, suggesting a possible role of enhanced EGP on the increase in fasting glucose (23,24). Although the basal EGP

in the present type 2 diabetic patients was not statistically higher than that in the control subjects, it should not be interpreted as normal in the face of their higher fasting glucose (23). EGP rate declined gradually reaching its nadir only at a time when total glucose concentration had already returned nearly to the basal level in the control subjects. A biphasic plasma insulin concentration was not reflected in the time course of EGP in the control subjects. Despite diminished insulin secretion during the first 20 min, EGP of the type 2 diabetic patients as a whole declined in a similar manner to the control subjects, and reached its nadir, which was comparable to that of the control subjects. These results suggest that the major factor to suppress EGP during the initial 20 min of FSIGT is glucose mass action, and the contribution of changes in plasma insulin concentration is minor. Also, the gradual suppression of EGP by acute hyperglycemia suggests that the suppression is mediated via an indirect pathway. Our observation is consistent with recent data, which reveal an important role of liver glucokinase in the regulation of EGP by glucose mass action (25).

Although the decline in EGP in the patients with type 2 diabetes seemed similar to that of the control subjects, it is likely that the glucose mass action to suppress EGP in the type 2 diabetic patients is less effective when higher levels of their

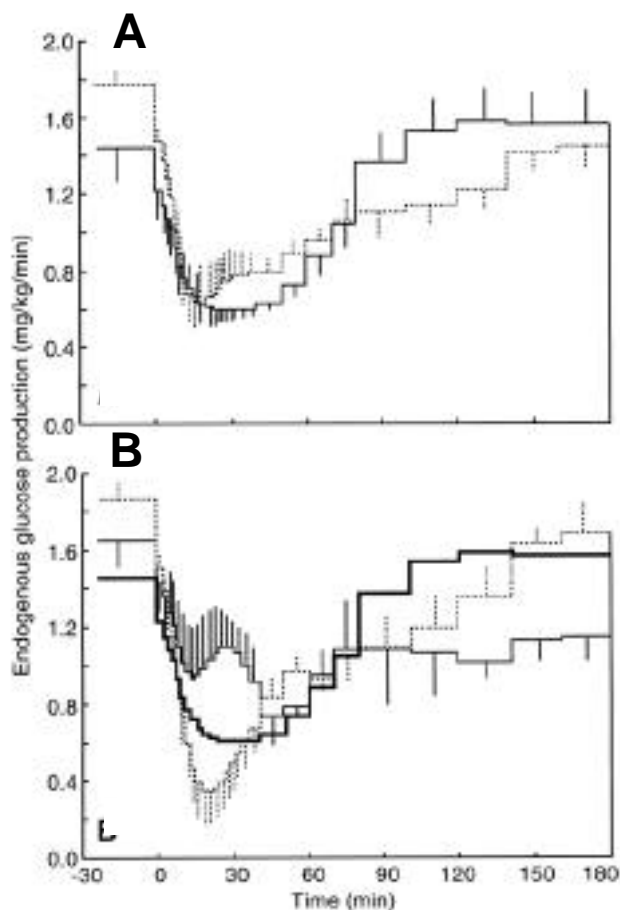


FIG. 3. Time course of endogenous glucose production derived from two-compartment minimal model and deconvolution. Values are means \pm SE for normal subjects ($n = 8$) (—) and type 2 diabetic patients ($n = 11$) (·····) (A). Mean of normal subjects ($n = 8$) (—) and means \pm SE for type 2 diabetic patients with higher ($n = 5$) (—) and lower ($n = 6$) (·····) HbA_{1c} level are shown in B.

plasma glucose throughout FSIGT is considered (23). When the differences in mean plasma glucose level at 30, 60, and 80 min were 49, 51, and 45 mg/dl ($P < 0.0001$, respectively), EGP was comparable between the two groups (Fig. 3A). Some but not all type 2 diabetic patients in the present study also showed blunted suppression of EGP during FSIGT. When EGP in the diabetic subjects was analyzed considering their recent glycemic control, the initial suppression was blunted in the patients with higher HbA_{1c} level. In diabetic subjects with poor glycemic control, excessively generated glucosamine is postulated to cause tissue insulin resistance (glucose toxicity) (26). Interestingly, glucosamine also has an inhibitory effect on liver glucokinase, which is involved in the regulation of EGP (25). Thus, glucosamine might be one of the candidates that causes blunted EGP suppression in the present diabetic subjects with higher HbA_{1c} levels.

After the suppression, EGP recovered to the basal level by 120 min in the control subjects, while that of the type 2 diabetic patients was still lower than the basal level at 180 min. The difference in the recovery process became evident during the 2nd hour of FSIGT, when plasma glucose concentration of the control subjects decreased below the basal level. These observations suggest that plasma glucose con-

centration declined to the level to elicit counterregulatory neurohormonal response in the control subjects, but not in the patients with type 2 diabetes. Exogenous additional insulin infused at 20–25 min seems to inhibit the recovery of EGP in the type 2 diabetic subjects, as they had still higher glucose level not to elicit a counterregulatory response. Alternatively, counterregulatory response to the decline in plasma glucose may be impaired in type 2 diabetic subjects (27). A role of counterregulatory response during FSIGT should be further examined in relation to the time course of EGP. The suppression of EGP was sustained to a greater extent in the subjects with higher HbA_{1c} level. The profile of plasma glucose and insulin was not different between the subjects with higher and lower HbA_{1c} levels (data not shown). Thus, direct and/or indirect action of insulin on the suppression of EGP may differ between these two groups. However, it seems difficult to selectively estimate insulin sensitivity related to EGP from the present results, because both S_p and S_I^* and S_I^{2*} had been already impaired to the same extent in the diabetic subjects.

Consistent with the previous studies (4–6), combined ability of glucose, per se, to increase glucose disposal and suppress EGP (S_G) of the type 2 diabetic patients in the present study was significantly lower than that in the control subjects. In contrast, the stimulatory effect of hyperglycemia on glucose disposal assessed by S_G^* and S_G^{2*} was not significantly impaired in the diabetic subjects. S_G showed no significant correlations with S_G^* and S_G^{2*} . One of the logical explanations for these findings is a possibility that a component of S_G to suppress EGP is impaired in the type 2 diabetic patients of this study. Although the decline in EGP in the patients with type 2 diabetes as a whole seemed similar to that of the control subjects, it might be less effective as discussed above. The suppression of EGP was apparently blunted in the patients with recent higher glycemia. Thus, blunted suppression of EGP might contribute to the decreased S_G in the diabetic subjects. Recent studies using the glucose clamp method (28,29) showed that EGP is normally suppressed by hyperglycemia, per se, in type 2 diabetic patients. However, the authors were cautious to draw strong conclusions, as basal EGP had been corrected by overnight insulin infusion in those studies (28,29). In fact, recent data reveal that overnight insulin infusion has a considerable effect on hepatic glucose handling (30). Further analysis is necessary to clarify whether the ability of glucose to suppress EGP is impaired in type 2 diabetic patients under their usual conditions, and its potential role on the decrease in S_G .

Recent reports have pointed out that the minimal model systematically overestimates S_G (10–13). Finegood et al. (11) compared the minimal model-derived S_G with model-independent measures. They demonstrated that the model-derived S_G is overestimated in dogs with a normal insulin profile, while it is correctly estimated in dogs with reduced insulin secretion. Subsequent analysis suggested that the use of a monocompartmental model to describe glucose kinetics may be responsible for inaccuracies in the estimation of model parameters (12,13,31). Two simulation studies (32,33) have examined the correlation between S_G and two-compartmental index of S_G , although the results were conflicting. Cobelli et al. (32) found a rather poor correlation between the two indexes. On the other hand, theoretical evaluation by Ni et al. (33) revealed that the minimal model-derived S_G is not equal to, but correlates well with actual glucose mass action. Analysis of the time

course of labeled glucose by a single-compartment (S_G^*) and two-compartment (S_G^{2*}) model, in the present study, provided similar estimates of peripheral tissue-specific glucose mass action, and confirmed the significant correlation between these two indexes.

Compared with the previous report (13), the precision of S_G^{2*} and S_I^{2*} in the present study was significantly improved, probably because of the additional insulin infusion. However, S_G^{2*} was still less precise than S_I^{2*} . In addition, two-compartment model configuration and additional two assumptions proposed by Caumo and Cobelli (16) were used unchanged to assess S_G^{2*} and S_I^{2*} ; glucose disposal from the central compartment was assumed to be three times greater than the disposal rate from the peripheral compartment, and R_{d0} was fixed to $1.0 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. In the only available study on R_{d0} in type 2 diabetic patients (29), the R_{d0} values were slightly greater in the type 2 diabetic patients, although HbA_{1c} levels of the patients ($8.9 \pm 0.5\%$) were higher than those of the present study. Underestimation of R_{d0} results in an increase in S_G^{2*} (see METHODS). Thus, it remains possible that the present study has failed to detect a subtle defect in the ability of glucose to promote its uptake in the type 2 diabetic subjects. On the other hand, we have confirmed that S_I^{2*} and the time course profile of EGP are robust against the changes in R_{d0} (data not shown).

In conclusion, we found that glucose mass action to stimulate its own uptake is not impaired in the lean Japanese patients with fairly controlled type 2 diabetes. This observation is different from a conclusion derived from S_G^* values of a labeled single-compartment minimal model study by Vicini et al. (34). S_G^* was significantly impaired in type 2 diabetic patients whose basal glucose ($214 \pm 36 \text{ mg/dl}$), insulin ($20 \pm 4 \mu\text{U/ml}$), and HbA_{1c} ($8.3 \pm 0.8\%$) were higher than those of the present study. Model-independent measures of glucose mass action on peripheral tissues were also impaired in type 2 diabetic patients with HbA_{1c} levels of 8–9% (28,29). Recent data also show that the glucose mass action is governed by a glucose concentration-dependent step (35). Taken together, currently available data suggest that glucose mass action to stimulate glucose uptake is finally impaired in the state of poor glycemic control, while the mass action to suppress EGP may be disturbed earlier, as well as secretion and action of insulin. Evidently, more clinical studies involving well-characterized patients with varying degrees of glucose intolerance are required to evaluate the importance of glucose mass action to stimulate its own uptake and suppress EGP in the pathogenesis of type 2 diabetes.

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REFERENCES

- Bergman RN: The minimal model: yesterday, today, and tomorrow. In *The Minimal Model Approach and Determinants of Glucose Tolerance*, Pennington Center Nutrition Series. Vol. 7. Bergman RN, Lovejoy JC, Eds., Baton Rouge, LA, Louisiana State University Press, 1997, p. 3–50
- Finegood DT: Application of the minimal model of glucose kinetics. In *The Minimal Model Approach and Determinants of Glucose Tolerance*, Pennington Center Nutrition Series. Vol. 7. Bergman RN, Lovejoy JC, Eds., Baton Rouge, LA, Louisiana State University Press, 1997, p. 51–122
- Best JD, Watanabe RM, Kahn SE, Ni T, Ader M, Bergman RN: Role of glucose effectiveness in the determinants of glucose tolerance. *Diabetes Care* 19:1018–1030, 1996
- Finegood DT, Hramiak IM, Dupre J: A modified protocol for estimation of insulin sensitivity with the minimal model of glucose kinetics in patients with insulin-dependent diabetes. *J Clin Endocrinol Metab* 70:1538–1549, 1990
- Taniguchi A, Nakai Y, Fukushima M, Kawamura H, Imura H, Nagata I, Tokuyama K: Pathogenic factors responsible for glucose intolerance in patients with NIDDM. *Diabetes* 41:1540–1546, 1992
- Taniguchi A, Nakai Y, Doi K, Fukushima M, Nagata I, Kawamura H, Imura H, Suzuki M, Tokuyama K: Glucose effectiveness in two subtypes within impaired glucose tolerance: a minimal model analysis. *Diabetes* 43:1211–1217, 1994
- Martin BC, Warram JH, Krolewski AS, Bergman RN, Soeldner JS, Kahn CR: Role of glucose and insulin resistance in development of type 2 diabetes: results of a 25-year follow-up study. *Lancet* 340:925–929, 1992
- Ader M, Pacini G, Yang YJ, Bergman RN: Importance of glucose per se to intravenous glucose tolerance: comparison of the minimal-model prediction with direct measurements. *Diabetes* 34:1092–1103, 1985
- Ward GM, Weber KM, Walters IM, Aitken PM, Lee B, Best JD, Boston RC, Alford FP: A modified minimal-model analysis of insulin sensitivity and glucose-mediated glucose disposal in insulin-dependent diabetes. *Metabolism* 40:4–9, 1991
- Quon MJ, Cochran C, Taylor SI, Eastman RC: Non-insulin-mediated glucose disappearance in subjects with IDDM: discordance between experimental results and minimal model analysis. *Diabetes* 43:890–896, 1994
- Finegood DT, Tzur D: Reduced glucose effectiveness associated with reduced insulin release: an artifact of the minimal-model method. *Am J Physiol* 271:E485–E495, 1996
- Caumo A, Vicini P, Cobelli C: Is the minimal model too minimal? *Diabetologia* 39:997–1000, 1996
- Vicini P, Caumo A, Cobelli C: The hot IVGTT two-compartment minimal model: indexes of glucose effectiveness and insulin sensitivity. *Am J Physiol* 273:E1024–E1032, 1997
- Avogaro A, Bristow JD, Bier DM, Cobelli C, Toffolo G: Stable-label intravenous glucose tolerance test minimal model. *Diabetes* 38:1048–1055, 1989
- Avogaro A, Vicini P, Valerio A, Caumo A, Cobelli C: The hot but not the cold minimal model allows precise assessment of insulin sensitivity in NIDDM subjects. *Am J Physiol* 270:E532–E540, 1996
- Caumo A, Cobelli C: Hepatic glucose production during the labeled IVGTT: estimation by deconvolution with a new minimal model. *Am J Physiol* 264:E829–E841, 1993
- Tsuruoka A, Matsuba I, Toyota T, Isshiki G, Nagasaki S, Ikeda Y: Antibodies to GAD in Japanese diabetic patients: a multicenter study. *Diabetes Res Clin Pract* 28:191–199, 1995
- Wolfe RR: *Radioactive and Stable-Isotope Tracers in Biomedicine: Principles and Practice of Kinetic Analysis*. New York, Wiley-Liss, 1992
- Cobelli C, Toffolo G, Bier DM, Nosadini R: Models to interpret kinetic data in stable isotope tracer studies. *Am J Physiol* 253:E551–E564, 1987
- Vicini P, Sparacino G, Caumo A, Cobelli C: Estimation of endogenous glucose production after a glucose perturbation by nonparametric stochastic deconvolution. *Comput Methods Programs Biomed* 52:147–156, 1997
- Overkamp D, Gautier JF, Renn W, Pickert A, Scheen AJ, Schumuller RM, Eggstein M, Lefebvre PJ: Glucose turnover in humans in the basal state and after intravenous glucose: a comparison of two models. *Am J Physiol* 273:E284–E296, 1997
- Press WH, Flannery BP, Teukolsky SA, Vetterling WT: *Numerical Recipes in Pascal*. Cambridge, Cambridge University Press, 1989
- Jeng C, Sheu WH, Fuh MM, Chen YI, Reaven GM: Relationship between hepatic glucose production and fasting plasma glucose concentration in patients with NIDDM. *Diabetes* 43:1440–1444, 1994
- Perriello G, Pampanelli S, Sindaco PD, Lalli C, Ciofetta M, Volpi E, Santusanio F, Brunetti P, Bolli GB: Evidence of increased systemic glucose production and gluconeogenesis in an early stage of NIDDM. *Diabetes* 46:1010–1016, 1997

25. Barzilai N, Hawkins M, Angelov I, Hu M, Rossetti L: Glucosamine-induced inhibition of liver glucokinase impairs the ability of hyperglycemia to suppress endogenous glucose production. *Diabetes* 45:1329–1335, 1996
26. Marshall S, Bacote V, Traxinger RR: Discovery of a metabolic pathway mediating desensitization of the glucose transport system: role of hexosamine biosynthesis in the induction of insulin resistance. *J Biol Chem* 266: 4706–4712, 1992
27. Cryer PE, Fisher JN, Shamon H: Hypoglycemia. *Diabetes Care* 17:734–755, 1994
28. Del Prato S, Matsuda M, Simonson DC, Groop LC, Sheehan P, Leonetti F, Bonadonna RC, DeFronzo RA: Studies on the mass action effect of glucose in NIDDM and IDDM: evidence for glucose resistance. *Diabetologia* 40:687–697, 1997
29. Basu A, Caumo A, Bettini F, Gelisio A, Alzaid A, Cobelli C, Rizza RA: Impaired basal glucose effectiveness in NIDDM: contribution of defects in glucose disappearance and production, measured using an optimized minimal model independent protocol. *Diabetes* 46:421–432, 1997
30. Wise SD, Nielsen MF, Cryer PE, Rizza RA: Overnight normalization of glucose concentrations improves hepatic but not extrahepatic insulin action in subjects with type 2 diabetes mellitus. *J Clin Endocrinol Metab* 83:2461–2469, 1998
31. Caumo A, Cobelli C, Finegood DT: Minimal model estimate of glucose effectiveness: role of the minimal model volume and of the second hidden compartment. *Am J Physiol* 271:E573–E576, 1998
32. Cobelli C, Vicini P, Caumo A: If the minimal model is too minimal, who suffers more: Sg or Si? *Diabetologia* 40:362–363, 1997
33. Ni T, Ader M, Bergman RN: Reassessment of glucose effectiveness and insulin sensitivity from minimal-model analysis: a theoretical evaluation of the single-compartment glucose distribution assumption. *Diabetes* 46:1813–1821, 1997
34. Vicini P, Avogaro A, Valerio A, Caumo A, Cobelli C: Glucose effectiveness, clearance rate and peripheral insulin sensitivity are impaired in NIDDM subjects: a hot minimal model study (Abstract). *Diabetes* 45:100A, 1996
35. Nielsen MF, Basu R, Wise S, Caumo A, Cobelli C, Rizza RA: Normal glucose-induced suppression of glucose production but impaired stimulation of glucose disposal in type 2 diabetes: evidence for a concentration-dependent defect in uptake. *Diabetes* 47:1735–1747, 1998