Glucose Processing During the Intravenous Glucose Tolerance Test

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The impact of the dynamic changes in plasma glucose and insulin levels observed during a frequently sampled intravenous (IV) glucose tolerance test (FSIGT) on whole-body glucose processing and muscle glycogen metabolism is not known. Paired randomized FSIGTs were performed in eight healthy subjects (age, 31 years; range, 28 to 35; BMI, 25.4 kg/m^2; range, 22.3 to 32.1), one with muscle biopsy samples and one without. The mean time average (0- to 40- and 0- to 120-minute) insulin levels during the test were 26.6 and 11.4 mU/l, respectively. Glucose oxidation increased following the IV gluclose bolus (basal 1.34 ± 0.21 *v* mean value at 0 to 120 minutes 2.09 ± 0.22 mg/kg fat-free mass [FFM]/min, $P < .02$). In contrast, fractional glucose-6-phosphate [G-6-P]) (0.1/10 mmol/L) skeletal muscle glycogen synthase activity in muscle biopsies obtained before and following the IV glucose bolus (−30, 30, 60, and 120 minutes, respectively) were unchanged (38.1% ± 3.3%, 38.3% ± 2.9%, 38.1% ± 2.3%, 35.4% ± 2.3%, NS). Skeletal muscle glycogen concentration decreased slightly (449 ± 54, 439 ± 55, and 383 ± 29, and 438 ± 48 mmol/kg dry weight, $P = .05$), indicating no net storage of glucose into glycogen during the FSIGT. G-6-P decreased (0.77 ± 0.08, 0.64 ± 0.07, 0.66 ± 0.07, and 0.54 ± 0.04 mmol/kg dry weight, $P < .05$). Levels of the insulin-regulatable glucose transporter, GLUT-4, were unchanged. Insulin sensitivity (Si), glucose effectiveness, and insulin secretion parameters (Ø1 and Ø2) were not affected by the muscle biopsy procedure. In conclusion, the FSIGT is associated predominantly with increased whole-body glucose oxidation with no apparent activation of muscle glucose storage as glycogen. Thus, the Si measured by the FSIGT, although similar in magnitude to the clamp-derived parameter, represents primarily glucose oxidation, in contrast to the euglycemic clamp, which involves glucose oxidation and storage.

THE EUGLYCEMIC-hyperinsulinemic clamp is considered the "gold standard" in the assessment of insulin sensitivity.[1,2] It is often combined with continuous indirect calorimetry for measurements of in vivo whole-body glucose and lipid oxidation rates,[3-5] and with infusion of labeled (3-^{3}H-glucose) glucose for quantification of hepatic glucose production.[2,6] Thus, in normal man during a hyperinsulinemic clamp with insulin levels at least 50 mU/L for at least 120 minutes, whole-body glucose oxidation and glucose storage (which includes nonoxidative glucose metabolism) increases, lipid oxidation decreases, and hepatic glucose production is inhibited.[1,5,6] If simultaneous muscle biopsies are performed during the clamp procedure and in vitro measurements of skeletal muscle glucose metabolism investigated, incorporation of glucose into skeletal muscle glycogen and insulin stimulation of the muscle enzyme, glycogen synthase, occurs.[2,7-9] The latter activation of the enzyme is tightly correlated with the increase in nonoxidative glucose metabolism.[7-9]

The frequently sampled intravenous (IV) glucose tolerance test (FSIGT) with subsequent minimal model analysis[10] is an alternate approach by which insulin sensitivity (Si) and glucose sensitivity (Sg), ie, the ability of glucose itself to promote glucose disposal at basal insulin (glucose effectiveness), can be estimated.[10-13] Furthermore, the insulin response to IV glucose (first- and second-phase insulin responsiveness, Ø1 and Ø2) can be estimated.[10,14] The FSIGT with minimal model analysis has gained widespread use in the last decade as a readily applicable test in investigations of whole-body glucose tolerance. The important question as to which metabolic pathways of glucose processing, namely glucose oxidation and/or glycogen storage, are activated during the FSIGT remains unknown.

Thus, the aim of the present study was to examine the impact of the dynamic changes in plasma insulin and glucose that occur during a FSIGT on whole-body glucose oxidation and skeletal muscle metabolism. Furthermore, we also examined the impact of the serial muscle biopsies on the minimal model parameters. To avoid the confounding use of tolbutamide injection during the FSIGT,[10,12] we used the minimal model analysis as implemented by the computer program Simulation Analysis and Modelling (SAAM) and a conversational interactive approach (CONSAM), which allows for simultaneous measurement of the model parameters without the necessity for tolbutamide.[13]

SUBJECTS AND METHODS

Subjects

Eight healthy male subjects without any family history of diabetes but with a range of body composition from lean to obese were studied (Table 1). The subjects were without any medication known to influence glucose metabolism.

Protocol

Before study entry, all subjects had an oral glucose tolerance test (OGTT) to establish normal glucose tolerance. After an overnight fast, a polyethylene catheter was inserted into the antecubital vein for blood sampling. Blood samples were obtained at basal and 2 hours after ingestion of 75 g glucose. Samples were analyzed for plasma glucose and insulin.

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Submitted May 27, 1995; accepted October 3, 1995.

Supported by grants from the Danish Diabetes Association, the Danish Research Council, the Diabetes Australia Research Foundation, the Clinical Research Institute, Odense University Hospital, the NOVO-Nordisk Foundation, and Direktør Jacob Madsen & Olga Madsens Fond. A.H. is a recipient of a research fellowship from the Weimann Foundation.

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0026-0495/96/4505-0010$03.00/0

Table 1. Clinical Characteristics of the Study Subjects

Characteristic	Value
No. of subjects (male/female)	8 (8/0)
Age (yr)	31 (28-35)
BMI (kg/m^2)	25.4 (22.3-32.1)
Weight (kg)	86.9 (68.0-113.6)
FFM (kg)	70.8 (52.3-85.4)
HbA_{1c} (%)	5.9 (5.2-6.7)
Basal glucose OGTT (mmol/L)	5.6 ± 0.9
Basal insulin OGTT (mU/L)	7.7 ± 1.8
2-hour glucose OGTT (mmol/L)	4.5 ± 1.0
2-hour insulin OGTT (mU/L)	16.8 ± 9.4

NOTE. Values are the mean ± SD or range.
Abbreviations: BMI, body mass index; HbA_{1c}, hemoglobin A_{1c}.

Two FSIGTs were performed in randomized order: one with muscle biopsies and one without. Subjects were requested to have a diet intake of more than 150 g carbohydrate/d for 3 days before the tests. The subjects were studied following a 10-hour overnight fast. Two polyethylene catheters were inserted into the antecubital veins. One catheter was used for IV glucose infusion; the other in the contralateral arm was used for blood sampling, and this hand and wrist were maintained in a heated plexiglas box during the entire study, to obtain arterialization of venous blood. A 300-mg/kg body weight glucose load (maximum, 25 g) was given intravenously over 1 minute as a 25% solution, immediately followed by 50-mL normal saline flush of the catheter.[12,13,15] 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, and 120 minutes in four of the subjects and at 140, 160, and 180 minutes in the remaining four subjects. Blood samples were stored at −20°C and plasma was subsequently analyzed from paired studies for glucose and insulin.

During both FSIGTs, continuous 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 at minute intervals for oxygen and carbon dioxide concentrations, and oxygen consumption and carbon dioxide production were calculated by the computer and recorded once per minute from the nonprotein respiratory quotient tables of Lusk[16] for the 120 minutes following the glucose load. After an equilibration period of 10 minutes, the average gas-exchange rate recorded 30 minutes before the IV glucose load was used to calculate rates of basal glucose and lipid oxidation as previously described.[4,17] After the IV glucose load, rates of glucose and lipid oxidation were calculated as the mean integrated oxidation rate for 15-minute intervals from the 1-minute estimates for a total of 2 hours. The protein oxidation rate was estimated from urinary nitrogen excretion (1 g nitrogen = 6.25 g protein) in urine collected over the test. Indirect calorimetry performed during non–steady-state conditions such as a FSIGT has potentially several limitations on the interpretation of data. These include unsteady respiration and CO_2 production rates, and utilization of pathways other than carbohydrate, lipid, and protein oxidation.[17-19] Given that continuous sampling of oxygen and carbon dioxide with minute-interval calculations of these parameters were taken for measurements of the integrated 15-minute glucose oxidation rate, it is unlikely that uneven respiration significantly impacted the present data.[5] Also, overnight-fasted subjects are not ketotic, and therefore, despite the large size of the CO_2 body pool (~40 L) compared with CO_2 production (~0.2 L/min), small changes in this pool are unlikely to have influenced the apparent minute volume of expired CO_2. Moreover, lactate production is relatively low during the IVGTT, and therefore, acute lactate-induced changes in pH are minor.[5,17,18] Finally, under the conditions of our experiments, gluconeogenesis from protein and lipogenesis from infused glucose, both of which can influence apparent CO_2 and O_2 uptake rates,[5,17] are also likely to be insignificant; gluconeogenesis is reduced by hyperglycemia and hyperinsulinemia, and lipogenesis is minimal with IV glucose infusion. In addition, the respiratory quotient remained less than 1 in all our study subjects. Thus, the use of continuous integrated indirect calorimetry during the FSIGT will yield valid rates of CO_2 production and glucose oxidation.

On the muscle biopsy day, muscle biopsies were taken from one vastus lateralis muscle using a Bergström needle (including suction) under local anesthesia.[20,21] Following the basal muscle biopsy at −30 minutes, three biopsies were performed at 30, 60, and 120 minutes. A total of three incisions were made, all in the same thigh. The 30- and 60-minute biopsies were taken from the same incision but with the needle pointing in different directions. Biopsies were rapidly (ie, <15 seconds) frozen in liquid nitrogen and stored at −80°C for later analysis.[15,20,21] The study was approved by the local ethics committee, and informed consent was obtained from all participants before testing.

Assays

Plasma glucose concentration was measured by the glucose oxidase method on a Beckman Glucose Analyzer (Beckman Instruments, 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, Uppsala, Sweden). The within-assay coefficient of variation was 5.6%, total assay variation 6.2%, and cross-reactivity with proinsulin 40%. Hemoglobin A_{1c} (HbA_{1c}) was estimated by high-performance liquid chromatography (normal range, 5.4% to 7.4%). Plasma lactate level was measured by a fluorometric method. Urinary nitrogen was calculated as reported by Tappy et al.[22]

The frozen muscle biopsies were freeze-dried and dissected free of blood, fat, and connective tissue. Glycogen level in the muscle biopsies was measured as glucose residues after hydrolysis with 1 mol/L HCl (at 100°C for 2 hours).[23] Concentrations of glucose-6-phosphate (G-6-P) were measured fluorometrically on neutralized perchloric acid extracts[23] as previously described from our laboratory,[2,20,21] and the data are expressed as millimoles per kilogram dry muscle weight. Glycogen synthase activity was measured as previously described.[8,9] Activity was measured without addition of G-6-P and in the presence of 0.05, 0.1, and 10 mmol/L G-6-P, the latter being taken as maximal activity of the enzyme. Activities are expressed as nanomoles of uridine diphosphate glucose (UDPG) incorporated into glycogen per minute per milligram extract protein. The total concentration of UDPG ([^{14}C]UDPG + cold UDPG) in the reaction mixture was 0.31 mmol/L. Protein content of the extract was determined by the method of Lowry et al.[24] Fractional velocities (FVs) of glycogen synthase were calculated as the ratio between the activities at 0.0 and 10 mmol/L G-6-P (FV0.0), 0.05 and 10 mmol/L G-6-P (FV0.05), and 0.1 and 10 mmol/L G-6-P (FV0.1).

GLUT-4 level was measured in the muscle powder of the frozen muscle biopsies (4.9 to 9.2 mg) that was obtained from homogenization of the muscle tissue for 7 seconds using a Polytron homogenizer (maximum setting; Professor P. Willems, Luzern, Switzerland) in 1.5 mL 50-mmol/L HEPES, pH 7.6, 250 mmol/L sucrose, 10 mmol/L EDTA, 1.5 mmol/L phenylmethylsulfonyl flouride (PMSF), and 400 kallikrein inhibitor units (KIU)/mL aprotinin.[25] A crude membrane fraction was isolated by a centrifugation procedure essentially as described previously.[25,26] The resulting pellet was resuspended in 150 μL homogenization buffer without

protease inhibitors, and protein content was determined by the Coomassie brilliant blue G-250 method using the kit from Bio-Rad (Richmond, CA). Protease inhibitors were added to the remaining crude membrane preparation (1.5 mmol/L PMSF and 400 KIU/mL aprotinin). Samples containing 15 μg protein from each crude membrane preparation were analyzed in duplicate by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting[25] followed by quantification of GLUT-4 by densitometric scanning of immunoreactivity. The antibody used for detection of GLUT-4 was affinity-purified polyclonal rabbit antibody directed against an artificial peptide identical to the 13 COOH-terminal amino acids of GLUT-4.

Calculations

Data from the FSIGT were analyzed using the minimal model of glucose disappearance[11] and insulin kinetics.[14] Glucose and insulin profiles were analyzed as formerly described using the program, Simulation Analysis and Modelling (SAAM), together with its interactive counterpart, CONSAM.[13,27] To enhance precision of the parameter estimates of the models, we used simultaneous analysis of the two FSIGTs[13,27] based on the minimal change postulate of Boston and Weber[28] or the principle of parsimony[29] as originally proposed by Berman,[30] to increase the ratio of number of observations (data points) to the number of parameters resolved. As discussed by these investigators, optimization of model estimatibility and physical meaning is achieved through multiple studies of single subjects exposed to a potential experimental perturbation.[28,30] In brief, (1) a model and its parameters is developed to characterize a kinetic system in a particular study (study 1 FSIGT without biopsies); (2) the state of the system is then potentially changed and some model parameters may need to be changed to bring the two models back into line (study 2 FSIGT with biopsies); (3) the smallest set of parameters required to describe that part of the model most affected by the perturbation are determined and used to characterize the system (paired analysis of studies 1 and 2).[13,30]

The minimal model of glucose disappearance yields two parameters; Si (in the model defined as P3/P2) and Sg (P1),[11] and two parameters of insulin secretion, Ø1 and Ø2.[14]

The IV glucose tolerance index (kg) was determined as the least-square slope of the line of the absolute glucose concentrations between 12 and 30 minutes after the glucose bolus. Fat-free mass (FFM) was calculated as the difference between body weight and total fat mass, the latter estimated by the bioimpedance method.[31]

In four of eight subjects, plasma glucose and insulin were only obtained for 2 hours. In the minimal model analysis of these latter FSIGTs, we used the basal values for plasma insulin and glucose as 180-minute estimates as described previously for children.[32] To establish the validity of this approach in our subjects, we separately reanalyzed four subjects who had the full 180-minute paired tests (ie, eight studies in all), using up to 120-minute time points and substituting the mean of the −30, −20, −10, and −1 glucose and insulin levels for the 180-minute value. In addition, we compared the difference between the "true" 180-minute concentrations of glucose and insulin with the "extrapolated" 180-minute (from basal values) concentrations, respectively.[32] The true and extrapolated Si values were, respectively, 5.40 ± 0.76 versus 5.66 ± 0.67 $min^{-1}/mU/L \times 10^{-4}$ (mean ± SEM, $P = .68$). Furthermore, true and extrapolated Sg values were, respectively, 1.89 ± 0.15 versus 1.73 ± 0.19 min^{-1} ($P = .10$). The fasting and 180-minute insulin and glucose concentrations were, respectively, 7.0 ± 0.6 versus 6.2 ± 0.4 mU/L ($P = .16$) and 5.3 ± 0.1 versus 4.9 ± 0.1 mmol/L ($P = .01$), as described previously.[32] Thus, despite the statistically lower 180-minute glucose concentration, we concluded that our method for estimating the model parameters using the 180-minute basal extrapolated level of glucose and insulin from the 120-minute studies was justified and did not introduce any biological bias in the calculated Si values.

Statistical Analysis

Results are presented as the mean ± SE. The Wilcoxon matched-pairs signed-rank test was used. In the analysis of measurements from the repeated muscle biopsies, we calculated the mean value from the post–IV glucose measurements and used this value as a summary measure,[33] which then was compared with the basal value using the Wilcoxon test. Correlation analyses were performed using Spearman rank-sum correlation analysis. Interday coefficient of variation was calculated from the formula, $100 \cdot \sqrt{(\Sigma(d/x)^2/2n)}$, where d is the difference between two measurements, x is the mean of these measurements, and n is the number of subjects. P values not greater than .05 were considered significant.

RESULTS

Comparison Between Days With and Without Biopsy

Fasting plasma glucose and insulin concentrations were similar between the day with and the day without muscle biopsy (Table 2 and Fig 1). Plasma glucose and insulin concentrations following the IV glucose bolus were virtually identical between the two experimental days (Fig 1). This is also apparent from the calculated IV glucose tolerance index, Kg (Table 2). Time-averaged insulin concentrations from 0 to 40 minutes and from 0 to 120 minutes were 26.6 ± 8.3 and 11.4 ± 3.5 mU/L (mean ± SD), respectively.

When FSIGT glucose and insulin data were subjected to the minimal model analysis, no difference was observed between Si, Sg, or first- and second-phase insulin responsiveness obtained from the days with and without muscle biopsy (Table 2). Acceptable intrasubject interday coefficients of variation of the various parameters were obtained (Table 2). However, as noted earlier, the minimal model analysis was performed using simultaneous analysis of the two FSIGTs to increase resolution of the minimal model parameters. As a consequence, P(1) Sg was equated in six of eight subjects. For this reason, no coefficients of variation have been calculated for this parameter. Individual differences between the Si obtained from the day with biopsy and the day

Table 2. Glucose and Insulin Kinetic Parameters Derived from the FSIGTs From Study Days With and Without Muscle Biopsies

	With Biopsy	Without Biopsy	CV (%)
Glucose (mmol/L)	5.34 ± 0.07	5.50 ± 0.14	5
Insulin (mU/L)	7.8 ± 0.6	7.4 ± 0.7	14
Kg ($10^{-2} \cdot min^{-1}$)	1.64 ± 0.20	1.58 ± 0.19	19
Si ($10^{-4} \cdot min^{-1} \cdot mU/L$)	5.37 ± 0.86	5.27 ± 0.58	26
Sg ($10^{-2} \cdot min^{-1}$)	1.55 ± 0.20	1.43 ± 0.19	
Ø1 ($mU/L \cdot min^{-1}$ per mg/dL)	3.56 ± 0.68	3.52 ± 0.68	20
Ø2 ($mU/L \cdot min^{-2}$ per mg/dL)	13.3 ± 4.0	13.0 ± 4.2	18

NOTE. Values are the mean ± SE. Glucose and insulin are mean values of 4 fasting measurements from each FSIGT.

Abbreviations: CV, interday CV; Kg, IV glucose tolerance index; Ø1, first-phase insulin responsiveness; Ø2, second-phase insulin responsiveness.

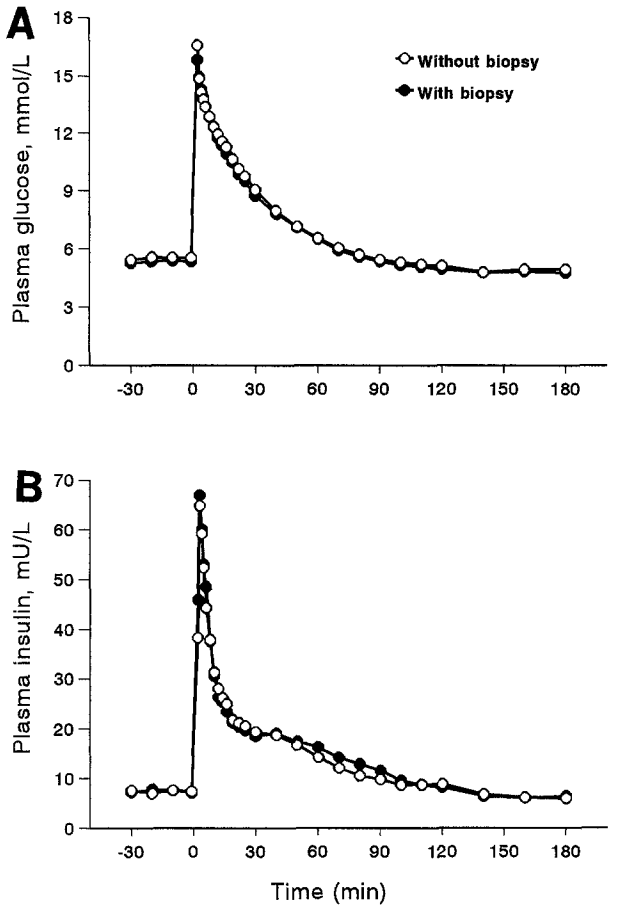

Fig 1. Mean plasma glucose (A) and insulin (B) profiles in 8 healthy subjects who underwent two FSIGTs: 1 day with 4 muscle biopsy samples, another day without muscle biopsy.

without biopsy are shown in Fig 2 and are small and randomly distributed.

Indirect Calorimetry

Glucose oxidation increased as a consequence of the IV glucose bolus (basal, 1.34 ± 0.21 *v* mean glucose oxidation 0 to 120 minutes, 2.09 ± 0.21 mg/kg FFM/min, $P < .02$) and lipid oxidation decreased (1.01 ± 0.13 *v* 0.78 ± 0.11 mg/kg FFM/min, $P < .02$; Fig 3). Moreover, no difference in glucose and lipid oxidation rates existed between the day with biopsy and the day without biopsy either in the basal rates (glucose, 1.34 ± 0.21 *v* 1.53 ± 0.16; lipid, 1.01 ± 0.13 *v* 0.99 ± 0.05 mg/kg FFM/min, $P = .40$ and .89, respectively) or in the post–glucose bolus rates (glucose, 2.09 ± 0.22 *v* 1.95 ± 0.18; lipid, 0.78 ± 0.11 *v* 0.86 ± 0.06 mg/kg FFM/min, $P = .67$ and .58, respectively).

Skeletal Muscle Glucose Processing

No increase in skeletal muscle glycogen concentration occurred during the FSIGT. In fact, absolute skeletal muscle glycogen concentrations decreased slightly, which was statistically significant (449 ± 54 *v* 420 ± 40 mmol/kg dry weight, basal *v* mean of post–IV glucose biopsies, $P = .05$; Table 3). No change in the activity of glycogen

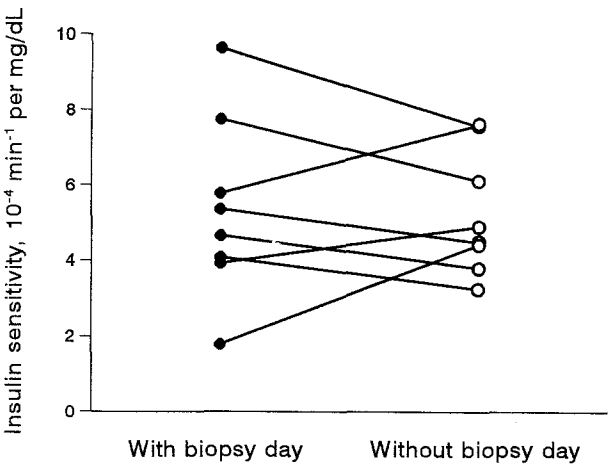

Fig 2. Individual changes in the Si obtained from the biopsy and nonbiopsy days in 8 healthy subjects. FSIGTs were performed in a randomized order.

synthase could be demonstrated in response to the IV glucose bolus either in the absolute activity or in the calculated FV (Table 3).

Following the IV glucose bolus, the concentration of

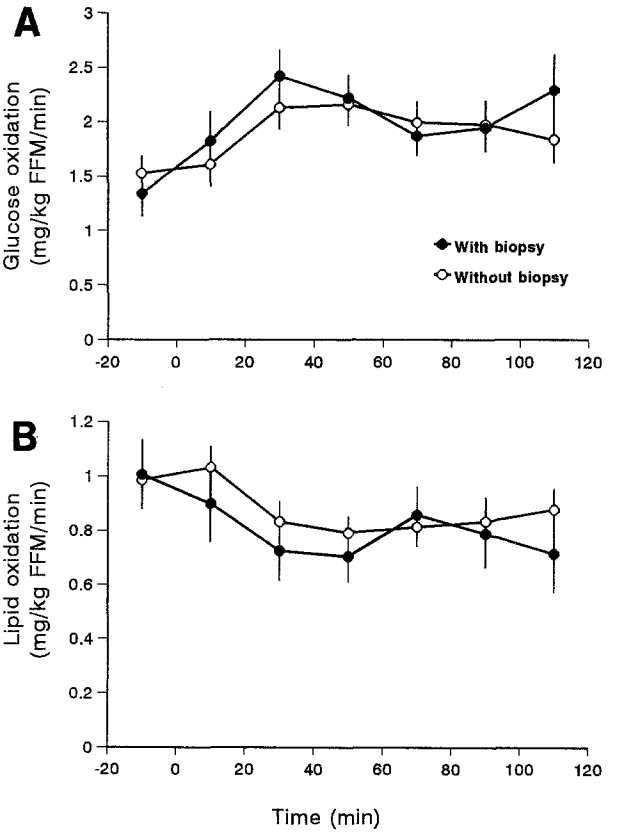

Fig 3. Glucose (A) and lipid (B) oxidation profiles in 8 healthy subjects who underwent two FSIGTs: 1 day with 4 muscle biopsy samples, another without muscle biopsy. The mean post–IV glucose bolus (0 to 120 minutes) glucose oxidation was higher ($P < .02$) and the mean lipid oxidation was lower ($P < .02$) than the basal oxidations. Values are the mean ± SEM.

Table 3. Activities of Skeletal Muscle Glycogen Synthase and Concentrations of Skeletal Muscle Glycogen at Basal and Following a Bolus of IV Glucose

Variable	Basal	30 min	60 min	120 min
G-6-P				
0.00	0.65 ± 0.12	0.59 ± 0.14	0.73 ± 0.17	0.51 ± 0.13
0.05	2.21 ± 0.41	1.95 ± 0.41	2.18 ± 0.41	1.55 ± 0.31
0.10	3.38 ± 0.61	2.95 ± 0.62	3.23 ± 0.41	2.37 ± 0.45
10.0	8.46 ± 1.21	7.22 ± 1.14	8.13 ± 1.16	6.50 ± 0.97
FV				
0.00/10	7 ± 1	8 ± 1	8 ± 1	8 ± 1
0.05/10	25 ± 2	25 ± 2	26 ± 2	23 ± 2
0.10/10	38 ± 3	38 ± 3	38 ± 2	35 ± 2
Glycogen	448 ± 54	439 ± 55	383 ± 30	438 ± 48

NOTE. Values are the mean ± SE. Activities of glycogen synthase are expressed as nmol UDP-glucose incorporation into glycogen/mg extract protein/min in the absence or in the presence of 0.05, 0.1, and 10 mmol/L of the allosteric activator, G-6-P. Fractional velocity (FV) is given as a percent. Glycogen is expressed as mmol/kg dry weight. The mean glycogen value post–IV glucose value (420 ± 40) is significantly decreased compared with baseline ($P = .05$).

G-6-P decreased (basal, 0.77 ± 0.08 *v* 0.64 ± 0.07, 0.66 ± 0.07, 0.54 ± 0.04, and 0.61 ± 0.04 mmol/kg dry weight (30-, 60-, and 120-minute biopsies and mean postglucose value, respectively, $P < .05$). Levels of the insulin-regulatable glucose transporter, GLUT-4, in skeletal muscle crude membrane preparations were unchanged following the IV glucose bolus (basal, 0.74 ± 0.07 *v* 0.70 ± 0.05, 0.71 ± 0.06, 0.70 ± 0.08, and 0.71 ± 0.05 arbitrary units (30-, 60-, and 120-minute biopsies and mean postglucose value, respectively, NS). No association could be demonstrated between the minimal model parameters and the glucose transporter, GLUT-4, or any other skeletal muscle parameters.

DISCUSSION

The present study demonstrates that the FSIGT is associated with increased whole-body glucose oxidation and inhibition of lipolysis. Skeletal muscle glucose storage as glycogen and activation of glycogen synthase did not occur in response to the acute IV glucose bolus, although a small decrease in intracellular G-6-P was noted. Frequently sampled muscle biopsies did not influence the various parameters of the minimal model, nor did they influence glucose or lipid oxidation rates.

Glucose oxidation rates increased by 55% in response to the IV glucose bolus, and this increase matched that seen at the end of a 2-hour euglycemic, low-dose (~20 mU/L) hyperinsulinemic clamp[9] and the OGTT.[34] Lipid oxidation was correspondingly decreased by approximately 23% during the FSIGT. These changes were accompanied by a decrease in skeletal muscle G-6-P concentration in response to the IV glucose bolus. Similar decreases in G-6-P following a hyperinsulinemic clamp and insulin-induced increases in glucose disposal, with or without hyperglycemia, have been reported in muscle biopsy samples taken at the end of such clamps by others.[2,35-37] Several reasons are possible. First, the increase in whole-body glucose oxidation following the IV glucose bolus with increased glucose disposal might indicate a finite size for the skeletal muscle intracellular G-6-P pool. Second, a possible inhibition of glycogenolysis by the FSIGT-associated hyperinsulinemia and hyperglycemia may tend to diminish the flux of G-6-P from glycogen to the muscle G-6-P pool.[38] Although we did observe a small (~3%) but statistically significant decrease in skeletal muscle glycogen concentration following the IV glucose bolus, this, together with the absent activation of glycogen synthase, would be consistent with a reduced glycogen synthesis/glycogenolysis coupling, and hence a net reduction in G-6-P flux to the muscle G-6-P pool from skeletal muscle glycogen.[38] However, these changes in muscle glucose metabolism during the FSIGT should not be extrapolated to the liver, where enhanced glycogen storage and decreased glycogenolysis is likely present. Thus, given that the mean infused glucose bolus in our subjects is 23.5 g and the mean increase in total whole-body glucose oxidation is 6.4 g, it can be calculated that about 17 g glucose must have been "stored" in the body. This storage during the combined hyperglycemia and hyperinsulinemia includes a combination of a decrease in hepatic glucose production,[39] an increase in hepatic glucose uptake with subsequent hepatic glycogen synthesis and decreased glycogenolysis,[40] and an increase in peripheral lactate production.[41]

The apparent lack of activation of the glycogen synthase enzyme and the absence of incorporation of glucose into skeletal muscle glycogen by insulin during the FSIGT are contrary to what is observed during a moderate to high hyperinsulinemic (50 to 100 mU/L) clamp technique, where in vivo activation of glycogen synthase and incorporation of glucose into glycogen occurs by the end (>120 minutes) of the clamp in nondiabetic subjects.[7,9,20] This steady-state situation with constant hyperinsulinemia and euglycemia for 2 to 3 hours is in striking contrast to the dynamic changes in plasma insulin and glucose observed during the FSIGT. The peak plasma insulin concentration (~70 mU/L), which, although similar to that seen during the clamp studies, only lasts for 5 minutes as opposed to several hours during a clamp procedure, and the averaged integrated insulin level over the FSIGT are considerably lower (~11 to 26 mU/L) for 30 to 45 minutes postglucose only, compared with that observed in the clamp studies.[9,20] When these facts are taken into account, the present results of no activation of glycogen synthase by the FSIGT are compatible with those found in very–low-dose 2-hour hyperinsulinemic clamps.[9,19] It is consistent with the insulin levels being less than the threshold for activation of glycogen synthase by insulin[9,19] and also applies to combined hyperglycemic–low-dose hyperinsulinemic clamp studies.[42,43] Thus, insulin-stimulated glucose disposal during an FSIGT is due to increased whole-body glucose oxidation, although a small undetectable net increase of in vivo glycogen deposition in muscle may occur with these dynamic changes in insulin and glucose. However, the small but significant decrease in muscle glycogen concentrations during the FSIGT would make this possibility unlikely.

What, then, does the minimal model–derived measure of insulin sensitivity, Si, represent? The present study demonstrates that the FSIGT reflects predominantly an increase in whole-body glucose oxidation, which contrasts with the

clamp where activation of glucose oxidation and glucose storage into glycogen occurs.[7-9] At low levels of insulin, transmembrane glucose transport[44-46] and/or glucose phosphorylation[18,47] are the rate-determining steps of insulin action, but at modest higher insulin levels, transmembrane glucose transport and intracellular glucose metabolic pathways both contribute to the net rates of insulin action and glucose disposal.[48-50] Importantly, with these increasing insulin levels from basal to modest hyperinsulinemia, increases in overall glucose disposal appear log-linear and represent changes in insulin activation of transmembrane glucose transport, glucose phosphorylation, and glucose oxidation, and activation of glycogen synthase with associated glucose storage.[9,19] Thus, at insulin levels of less than about 100 mU/L, insulin-induced incremental increases of net glucose disposal are similar, which means that measurements of Si are comparable within this insulin range regardless of the underlying insulin-induced metabolic processes occurring. Therefore, Si as measured by the FSIGT reflects transmembrane glucose transport and glucose phosphorylation and glucose oxidation, whereas during the physiologic hyperinsulinemic clamp, the Si measurement reflects these metabolic processes, as well as activation of glycogen synthase. In other words, normal insulin-sensitive individuals tested below the threshold of insulin activation of glycogen synthase, glucose oxidation, and transmembrane glucose transport are stimulated, with a corresponding stimulation of the oxidative glycolytic pathway and decrease of intracellular G-6-P concentrations. Thus, Si values measured by the FSIGT and clamp methods reflect equivalent measures of the rate of glucose disposal but may represent different underlying metabolic aspects of net insulin action.

The content of GLUT-4 protein in the crude membrane fractions was identical in the four biopsies obtained during the FSIGT, which contrasts with the reported decrease in GLUT-4 content observed in normal subjects during a prolonged (>3-hour) modest hyperinsulinemic clamp.[51,52] This latter observation during clamp studies is suggested to be due to a direct effect of tonic hyperinsulinemia on GLUT-4 turnover,[51,52] a situation that clearly does not hold for a dynamic FSIGT study. It is also noted that the coefficient of variation (CV) was low among biopsies from individual subjects, indicating a high reproducibility of the GLUT-4 assay method used (mean CV, 11.2%, range, 1.4% to 21.7%). However, given the limitations of measurements of total GLUT-4 content in crude membrane preparations, it is not possible to clarify the role of GLUT-4 in the various parameters of the FSIGT minimal model analysis.

In theory, multiple muscle biopsies could influence measurements of Si, Sg, and insulin secretion by promoting adrenaline, cortisol, and/or growth hormone responses to the biopsy procedures.[53] When we directly compared the influence of the muscle biopsy procedures on FSIGT by performing two FSIGTs, one with four serial muscle biopsies and one without any muscle biopsies, no difference was found between the minimal model parameters for the two FSIGTs. This is also apparent visually from the plasma insulin and glucose profiles. Moreover, the interday CV of the insulin response to IV glucose was comparable to that of previous reports.[54,55] The intrasubject CV of the Si value seemed higher in the present study compared with previous studies,[54,56] but this was due to one person with a halving of the Si value during the biopsy study day compared with the nonbiopsy day. If this subject is excluded from the calculation, the CV of Si decreases to 16%, which is close to the CVs from previous reports.[54,56] In contrast to the present study, a recent study using the hyperinsulinemic glucose clamp technique with no biopsy or a single biopsy taken just before the clamp study found a small 12% statistically significant decrease in insulin action on the biopsy day.[53] However, inspection of their data reveals that insulin-induced glucose disposal decreased in six of eight subjects, and these were from the most insulin-sensitive individuals.[53] In our subjects, the change in Si on the biopsy day was not related to the initial insulin sensitivity. Together, these data indicate that the muscle biopsy procedure, although not always completely painless, can be performed with only minor or no alterations of in vivo biological insulin action and insulin secretion in most individuals.

In conclusion, the IVGTT with dynamic changes of plasma insulin and glucose is associated predominantly with increased oxidative glucose metabolism and with no apparent activation of glucose storage and glycogen synthase. Si measured by the FSIGT therefore represents insulin's action on transmembrane glucose transport and whole-body glucose oxidation of the individual.

ACKNOWLEDGMENT

The authors would like to acknowledge the expert technical assistance of Henny Hansen, Henriette Vorup, Karin Dyregaard, Charlotte Fage Larsen, Bente Hansen, and Karin Clante.

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