Effects of Metformin on the Pathways of Glucose Utilization After Oral Glucose in Non–Insulin-Dependent Diabetes Mellitus Patients

Françoise Féry, Laurence Plat, and Edmond O. Balasse

To analyze the effects of metformin (M) on the kinetics and pathways of glucose utilization after glucose ingestion, nine non–insulin-dependent diabetes mellitus (NIDDM) patients underwent two 5-hour oral glucose tolerance tests (OGTTs) preceded in random order by a 3-week treatment with either M (850 mg twice per day) or placebo. Each test included intravenous infusion of 3-^{3}H-glucose and labeling of the oral dose (75 g) with 1-^{14}C-glucose, with measurements of glucose kinetics, glycolytic flux ($^{3}H_2O$ production), and glucose oxidation (indirect calorimetry and expired $^{14}CO_2$). Basal glycemia was decreased by M (6.6 *v* 8.2 mmol/L, $P < .01$) with no changes in insulin levels, with the hypoglycemic effect correlating strongly ($P < .001$) with a decrease in glucose production. Mean 0- to 5-hour postprandial glycemia was also decreased by the drug (9.9 *v* 12.2 mmol/L, $P < .04$), lactate concentration was increased (1.79 *v* 1.44 mmol/L, $P < .01$), and absolute insulin levels were increased, but not to a significant extent. The rates of appearance (Ra) of exogenous and endogenous glucose were not modified, and the hypoglycemic effect of M in the postprandial state was entirely related to an increase in systemic glucose disposal (85.1 *v* 77.5 g/5 h, $P < .001$). Carbohydrate oxidation was unchanged, and glycolytic flux and nonoxidative glycolysis were increased by approximately 13 g/5 h ($P < .01$), with the excess lactate produced probably being converted to glycogen in the liver. Whole-body glycogen synthesis through the direct pathway tended to be reduced (−8 g/5 h, $P > .05$). Thus, M decreases postprandial glycemia by increasing glucose disposal and stimulates lactate production. The data also suggest that the drug increases the proportions of glycogen deposited through the indirect rather than the direct pathway.

METFORMIN (M) is a biguanide molecule used in many countries, including Europe and Canada, for the treatment of non–insulin-dependent diabetes mellitus (NIDDM). It has been approved only recently by the US Food and Drug Administration. Despite intensive research for more than 25 years, its mode of action remains poorly understood, although there is general agreement that it does not involve stimulation of insulin secretion.[1] In addition to its antihyperglycemic effect, the drug tends to increase lactate concentration, but clinical cases of lactic acidosis are rare,[2] and M has been shown to be a safe drug if properly administered.[3] Depending on the experimental protocol used to explore its mechanism(s) of action, the liver,[4,5] intestine,[6,7] or peripheral tissues[8,9] have been pointed out as the main target of M action. Regarding peripheral tissues, use of the euglycemic-hyperinsulinemic clamp combined with indirect calorimetry has demonstrated that M improves insulin-mediated glucose disposal in the nonoxidative component,[10-12] an effect that tends to correct a fundamental defect in peripheral insulin action in NIDDM,[13-15] but it has not been established in humans which of the two components of nonoxidative glucose disposal (lactate production or glycogen synthesis) is specifically stimulated by the drug.

The present study performed in NIDDM patients aims to analyze the effects of a 3-week M treatment on glucose metabolism during an oral glucose tolerance test (OGTT). The methodology includes the use of a dual isotope and indirect calorimetry. The unanswered questions regarding the effect of M on lactate production and glycogen synthesis were specifically addressed through quantification of the glycolytic flux based on measurement of $^{3}H_2O$ production from 3-^{3}H-glucose.

SUBJECTS AND METHODS

Patients

Nine obese male NIDDM patients (age, 50.1 ± 2.8 years; weight, 81.3 ± 3.9 kg; height, 170 ± 4 cm) participated in the study. The known duration of diabetes was 6.7 ± 2.0 years. The patients had no disease other than diabetes, except for two: one had hypertension treated with enalapril, and the other had a depressive disorder treated with amitriptyline. These therapies were maintained unchanged throughout the study period. At the time of recruitment, five patients were being treated with diet alone, three with M, and one with the sulfonylurea drug gliquidone. Antidiabetic medication was discontinued at least 3 weeks before the patients entered the study. The nature, purpose, and potential risks of the study were explained to the patients, and their informed consent was obtained before participation. The protocol was approved by the Ethics Committee of the Faculty of Medicine of the University of Brussels.

Experimental Protocol

Each patient underwent two OGTTs at 3- to 4-week intervals. The tests were preceded in random order by a 3-week treatment with either M (850 mg twice per day) or placebo. Four patients started on M and five on placebo. Patients were instructed not to change their usual diet during the course of the study.

On each experimental day, the study was started at 7 AM and subjects remained in a semirecumbent position throughout the test. A primed, constant infusion of 3-^{3}H-glucose (NEN, Boston, MA) was administered through an antecubital vein for 7.5 hours. The priming dose was 120 to 150 times the amount infused per minute depending on the degree of hyperglycemia. A period of 150 minutes was allowed for equilibration of the tracer in the glucose pool. At the end of the basal period, patients ingested a last tablet of placebo or 850 mg M, immediately followed by a 75-g glucose load dissolved in 320 mL flavored water and labeled with 100 μCi 1-^{14}C-glucose (NEN). Glucose concentration and specific activity of the oral load were determined for each test. Arterialized venous blood was obtained from a dorsal vein of the hand opposite to the infusion side. The hand was maintained in an

From the Department of Endocrinology, Erasmus Hospital, Brussels; and the Laboratory of Experimental Medicine, University of Brussels, Brussels, Belgium.

Submitted May 30, 1996; accepted August 20, 1996.

Supported by grants from LIPHA Belgium and the Fonds de la Recherche Scientifique Médicale Belge (3.4578.92).

Address reprint requests to Françoise Féry, MD, Laboratory of Experimental Medicine, University of Brussels, 808 Route de Lennik, B-1070 Brussels, Belgium.

0026-0495/97/4602-0021$03.00/0

electric heating pad to provide adequate arterialization of the venous blood as indicated elsewhere.[16] Samples were obtained every 15 minutes during the last 45 minutes of the basal period and every 30 minutes for the remaining 5 hours, with the patency of the vein maintained between samples with a slow drip of saline. Subjects voided before the start of the test, and urine was collected at the end of the experiment. Respiratory gas exchanges were measured during the basal period and for 15-minute periods every 30 minutes during the 5 hours after glucose ingestion using a Deltatrac Metabolic Monitor (Datex, Helsinki, Finland) equipped with a ventilated hood. Every hour after glucose ingestion, a sample of expired air was collected in a Douglas bag for measurement of CO_2 specific activity.

Approximately 3 weeks after the second OGTT, the patients underwent a measurement of total-body water volume. Therefore, a basal blood sample was obtained for evaluation of residual ^3H_2O content, followed by intravenous administration of a known amount (~80 μCi) of ^3H_2O (NEN). Blood samples were then collected 2, 2.5, and 3 hours after injection of the tracer for determination of the ^3H_2O content of plasma water.

Analytical Procedures

Blood samples were collected in heparinized syringes and transferred to tubes kept on ice. The samples for measuring unlabeled and labeled glucose, lactate, and alanine concentrations contained NaF. After centrifugation at 4°C, plasma was stored at −20°C until assayed. Plasma glucose was determined using a glucose oxidase method (Test Combination Glucose; Boehringer, Mannheim, Germany). Plasma ^3H-glucose and ^3H_2O were determined after deproteinization by the Somogyi method. ^3H-glucose was counted by dual-scintillation spectrometry on evaporated filtrates reconstituted with water. ^3H_2O was determined as the difference between tritium counts obtained with and without evaporation. ^3H_2O in plasma water was calculated[17] by dividing its concentration in total plasma by 0.93. Except for deproteinization, glucose, ^3H-glucose, and ^3H_2O in urine were determined as for plasma. To determine the amount of ^{14}C present in the first carbon of glucose, aliquots of the plasma samples were processed with a fermentation method using *Leuconostoc mesenteroides* as described previously.[16,18] Lactate and alanine were determined on a neutralized perchloric filtrate of plasma using standard enzymatic methods.[19] Free fatty acids (FFAs) (NEFA; WAKO, Neuss, Germany) and urea (Urea Merckotest; Merck, Darmstadt, Germany) were assayed with enzymatic methods. Plasma insulin,[20] C-peptide (RIA-Mat C-Peptide; Byk-Sangtec Diagnostic, Dietzenbach, Germany), and glucagon[21] were determined by radioimmunoassay. Total urinary nitrogen was assayed with the Kjeldahl method using a Kjeltec 1 apparatus (Tecator, Höganäs, Sweden). To measure $^{14}CO_2$ specific activity in expired air, 3 mL of a solution of Hyamine (Rohm & Haas, Philadelphia, PA) in methanol (0.33 mol/L) was placed in 10-mL counting vials, and expired air from the Douglas bags was pumped slowly through the solution until neutralization in the presence of phenolphthalein. The vials were then counted after addition of scintillation fluid. For each experiment, the Hyamine solution was titrated with HCl before use. All determinations were made in duplicate, except for plasma ^3H_2O content at 0 and 300 minutes, which was measured in quadruplicate.

Calculations

Total-body water volume was calculated from the ratio between the amount of ^3H_2O injected (dpm) and the steady-state concentration of ^3H_2O in plasma water (mean of three determinations obtained between 2 and 3 hours postinjection).

Rates of total systemic glucose appearance (Ra) and disappearance (Rd) were calculated for each period between two consecutive samples from the ^3H-glucose infusion rate and the ^3H-glucose data in plasma using the non–steady-state equations of Steele.[22] For these calculations, it was assumed that the functional volume of distribution of glucose represented 13% of body weight. Tissular Rd was calculated as the difference between total Rd and urinary glucose loss. Urinary loss corresponding to each period (Fig 1) was calculated from the measured amount of glucose excreted during the 5-hour OGTT assuming glycosuria only occurred at levels greater than 10 mmol/L and was proportional to the glucose concentration above this level. The metabolic clearance rate (MCR) of glucose was calculated as the ratio between tissular Rd and the corresponding mean glucose concentration. The 1-^{14}C-glucose data were used to calculate the Ra of oral glucose. Therefore, the contribution to plasma glucose concentration made by

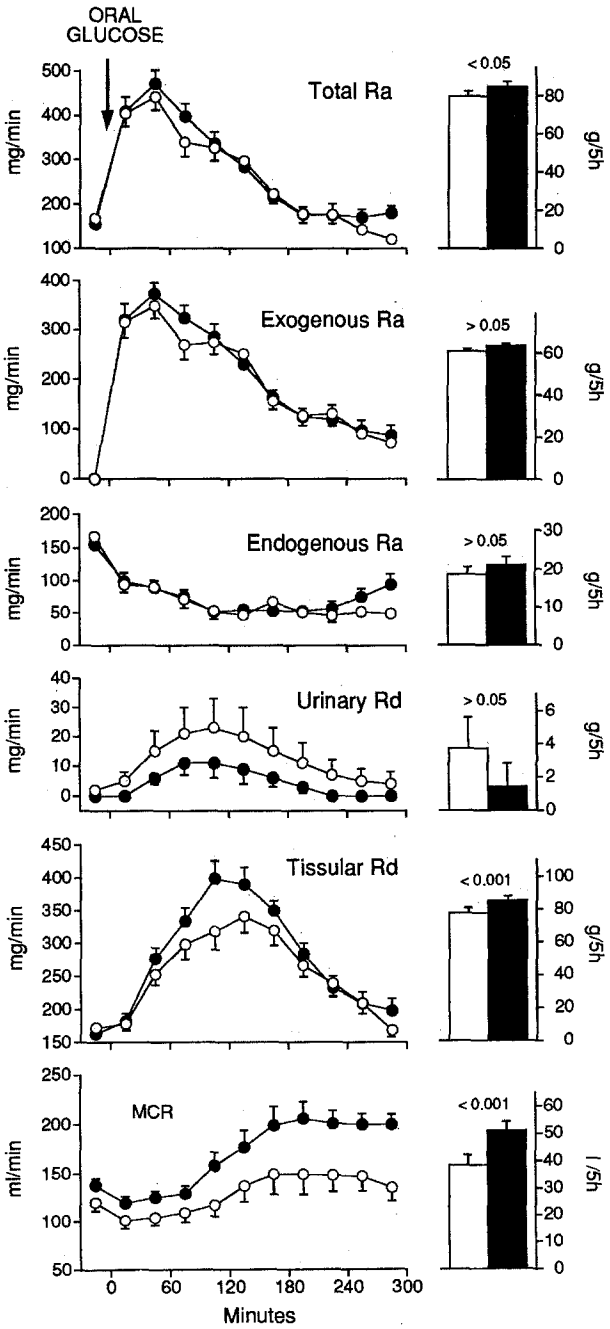

Fig 1. Glucose kinetics during an OGTT in NIDDM patients (N = 9). Columns represent cumulated values for the 0- to 5-hour period. (○, □) Placebo; (●, ■) M.

ingested glucose was estimated by dividing the plasma concentration of 1-^{14}C-glucose by the ^{14}C specific activity of the glucose drink. With the use of this calculated glucose concentration of exogenous origin and the measured ^{3}H-glucose counts, the exogenous Ra was estimated as indicated earlier for total Ra. The Ra of endogenous glucose was subsequently obtained as the difference between the Ra of total and oral glucose. Assuming that intestinal absorption of the oral load was completed after 5 hours,[23] the difference between the amount of ingested glucose and exogenous Ra represents first-pass splanchnic glucose extraction.[23] Since tissular Rd corresponds to glucose taken up only from the systemic circulation, whole-body total glucose uptake was calculated as the sum of tissular Rd and first-pass splanchnic glucose uptake.[24] The uptake of oral glucose (grams per 5 hours) was obtained by subtracting from the oral load the amount of glucose lost in urine and that remaining in the glucose pool.

Glycolytic flux and glycogen synthesis for the entire 5-hour OGTT period were calculated according to the following equations:

$$^{3}H_2O \text{ production (dpm/5 h)} = [\Delta_{0\text{-}5h} \text{ concentration of } ^{3}H_2O \text{ (dpm/mL)} \times \text{body water (ml)}] + {}^{3}H_2O \text{ eliminated in urine (dpm/5 h)}, \quad (1)$$

$$^{3}\text{H-glucose uptake (dpm/5 h)} = {}^{3}\text{H-glucose infused (dpm/5 h)} - {}^{3}\text{H-glucose eliminated in urine (dpm/5 h)} - \Delta_{0\text{-}5h}\ {}^{3}\text{H-glucose extracellular pool (dpm/5 h)}, \quad (2)$$

$$\text{Fraction of glucose taken up undergoing glycolysis} = \text{Eq 1/Eq 2}, \quad (3)$$

$$\text{Whole-body glycolytic flux (g/5 h)} = \text{whole-body glucose uptake (g/5 h)} \times \text{Eq 3}, \quad (4)$$

$$\text{and whole-body glycogen synthesis (g/5 h)} = \text{whole-body glucose uptake (g/5 h)} - \text{Eq 4}. \quad (5)$$

The mode of calculation of $^{3}H_2O$ production was validated in separate experiments on six normal subjects who were submitted to the protocol used in the placebo group, except that 2-^{3}H-glucose (which loses ^{3}H as $^{3}H_2O$ in the hexose-isomerase reaction) was substituted for 3-^{3}H-glucose. Under these conditions, we found that the amount of $^{3}H_2O$ generated over 5 hours of the OGTT represented 97% ± 2% of the amount of 2-^{3}H-glucose taken up by tissues during the same period.

Total carbohydrate and lipid oxidation rates were calculated[25] from CO_2 production, O_2 consumption, and urinary nitrogen output corrected for the changes in urea pool size, assuming that the volume of distribution of urea represents 60% of body weight.[26] Nonoxidative glucose disposal was calculated over the 5-hour period as the difference between the uptake of oral glucose and carbohydrate oxidation measured by indirect calorimetry. Oxidation of oral glucose was calculated as the ratio between the 0- to 5-hour cumulated $^{14}CO_2$ production and the specific activity of the glucose load. No attempt was made to correct the data for $^{14}CO_2$ retention in the bicarbonate pool or in other metabolic pools of the body.

Statistical Analysis

All values are presented as the mean ± SEM. The comparison between the two treatment periods considered the mean concentrations and fluxes for the basal period and mean concentrations and cumulated fluxes for the 0- to 5-hour postprandial period. No attempt was made to compare the time course of changes in concentrations or fluxes under the two experimental conditions. All comparisons were made using a two-tailed paired *t* test. Relationships between variables were analyzed by simple correlations. *P* less than .05 was considered significant.

RESULTS

Basal State

The effects of M on substrate and hormone concentrations and on glucose kinetics in the basal state are displayed in Table 1. M decreased the glucose concentration (6.6 ± 0.3 *v* 8.2 ± 0.7 mmol/L, $P < .01$), with the magnitude of the effect correlating strongly with the initial glucose level ($r = .92$, $P < .001$). Glucose production was inhibited by the drug, but not to a significant extent (155 ± 10 *v* 167 ± 11 mg/min, $P > .05$); however the decrease in glycemia correlated significantly with the decrease in Ra ($r = .83$, $P < .01$). The MCR of glucose was stimulated (130 ± 7 *v* 112 ± 9 mL/min, $P < .001$), and the change in the MCR correlated negatively with the change in glucose concentration ($r = -.71$, $P < .05$). M had no significant effect on insulin, C-peptide, glucagon, lactate, alanine, or FFA levels. Carbohydrate oxidation (74 ± 11 *v* 82 ± 15 mg/min, $P > .05$) and lipid oxidation (65 ± 9 *v* 68 ± 11 mg/min, $P > .05$) were unaffected by the drug.

OGTT

The time courses of substrate and hormone levels and glucose fluxes are depicted in Figs 1 and 2 for both treatment periods. Oral glucose tolerance was improved after M (Fig 2), with mean postglucose levels being decreased by approximately 20% ($P < .04$). As for basal glycemia, the amplitude of the hypoglycemic effect of M was strongly correlated with the mean postprandial levels recorded in the absence of the drug ($r = .91$, $P < .001$). Mean insulin and C-peptide levels were slightly more elevated after M, but not to a significant extent (Fig 2). Mean glucagon levels were unchanged (233 ± 31 *v* 234 ± 31 ng/L, $P > .05$). Mean 0- to 5-hour lactate (1.79 ± 0.15 *v* 1.44 ± 0.11 mmol/L, $P < .01$) and alanine (320 ± 20 *v* 280 ± 20 μmol/L, $P < .02$) concentrations were increased after M.

The Ra of exogenous and endogenous glucose in the systemic circulation was not significantly altered by drug treatment, although inhibition of hepatic glucose output tended to be of shorter duration (Fig 1). After M treatment, the amount of total glucose (endogenous + exogenous) appearing in periph-

Table 1. Metabolic and Hormonal Effects of M in the Fasting State

Parameter	Placebo	M	*P*
Plasma concentration			
Glucose (mmol/L)	8.2 ± 0.7	6.6 ± 0.3	<.01
Insulin (pmol/L)	114 ± 12	90 ± 12	>.05
C-peptide (nmol/L)	0.99 ± 0.10	0.89 ± 0.10	>.05
Glucagon (ng/L)	234 ± 31	233 ± 31	>.05
Lactate (mmol/L)	1.16 ± 0.09	1.09 ± 0.09	>.05
Alanine (μmol/L)	260 ± 20	280 ± 20	>.05
FFA (μmol/L)	550 ± 70	510 ± 50	>.05
Glucose metabolism			
Ra (mg/min)	167 ± 11	155 ± 10	>.05
Urinary Rd (mg/min)	2 ± 2	0 ± 0	>.05
Tissular Rd (mg/min)	165 ± 12	155 ± 10	>.05
MCR (mL/min)	112 ± 9	130 ± 7	<.001
Carbohydrate oxidation (mg/min)	82 ± 15	74 ± 11	>.05

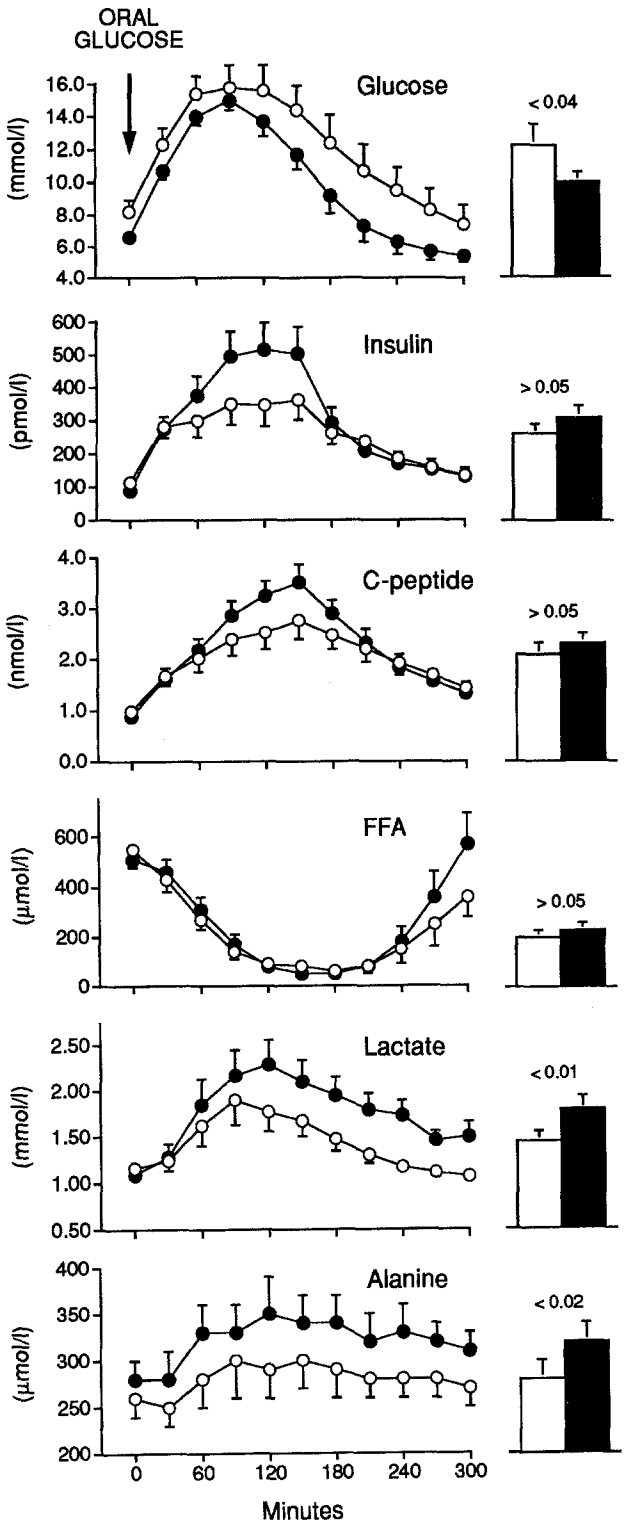

Fig 2. Plasma concentrations of substrates, insulin, and C-peptide during an OGTT in NIDDM patients (N = 9). Columns represent mean values for the 0- to 5-hour period. (○, □) Placebo; (●, ■) M.

eral blood was slightly more elevated (84.7 ± 2.6 *v* 79.6 ± 2.7 g/5 h, $P < .05$). The tissular Rd (85.1 ± 2.8 *v* 77.5 ± 3.3 g/5 h, $P < .001$) and MCR (51 ± 3 *v* 38 ± 4 L/5 h, $P < .001$) were significantly enhanced. Total whole-body glucose uptake (which includes tissular Rd and first-pass splanchnic uptake) was also significantly stimulated by the drug (Table 2).

Increases in plasma water specific activity are depicted in Fig 3 for both the placebo and M experiments. As expected, the curves are not strictly linear, with the smallest slope being observed during the initial 2 hours following glucose administration, at a time when glucose concentration is the highest and ^{3}H-glucose specific activity the lowest.

The effect of M on the pathways of glucose utilization during the OGTT are depicted in Table 2. M enhanced the glycolytic flux (66.3 ± 5.5 *v* 52.6 ± 3.6 g/5 h, $P < .01$), due to the combination of a stimulation of whole-body glucose uptake (95.9 ± 2.4 *v* 90.2 ± 3.1 g/5 h, $P < .01$) and an increase in the percentage of glucose undergoing glycolysis (69% ± 4% *v* 58% ± 3%, $P < .02$). Glycogen synthesis was reduced, but not to a significant extent. Carbohydrate oxidation (41.7 ± 2.3 *v* 42.4 ± 3.5 g/5 h, $P > .05$) and lipid oxidation (14.7 ± 2.2 *v* 15.3 ± 2.3 g/5 h, $P > .05$) were unaffected by M. Nonoxidative glucose disposal was slightly and nonsignificantly enhanced (34.3 ± 1.7 *v* 29.6 ± 2.9 g/5 h, $P > .05$). Oxidation of oral glucose uncorrected for $^{14}CO_2$ retention (and therefore significantly underestimated) was, respectively, 9.7 ± 0.5 and 9.5 ± 0.7 g/5 h in the treated versus placebo groups ($P > .05$).

Body weight (81.3 ± 4.0 *v* 81.3 ± 3.9 kg), glycosylated hemoglobin (6.0% ± 0.3% *v* 5.9% ± 0.3%), and plasma concentrations of total cholesterol (4.53 ± 0.28 *v* 4.86 ± 0.21 mmol/L), high-density lipoprotein cholesterol (1.14 ± 0.16 *v* 1.19 ± 0.16 mmol/L), and triglycerides (1.11 ± 0.15 *v* 1.10 ± 0.12 mmol/L) were not different after M versus placebo.

DISCUSSION

Three weeks of treatment with M in NIDDM patients significantly modified glucose metabolism both in the fasting

Table 2. Effects of M on Pathways of Glucose Utilization During an OGTT

Glucose Metabolism	Placebo	M	*P*
(a) Ingested glucose	73.8 ± 0.6	74.4 ± 0.4	>.05
(b) Uptake of oral glucose	72.0 ± 3.5	76.0 ± 1.0	>.05
(c) Systemic appearance of oral glucose	61.0 ± 1.4	63.7 ± 1.1	>.05
(d) Initial splanchnic extraction (a − c)	12.7 ± 1.0	10.8 ± 0.9	>.05
(e) Systemic glucose disposal	77.5 ± 3.3	85.1 ± 2.8	<.001
(f) Whole-body glucose uptake (d + e)	90.2 ± 3.1	95.9 ± 2.4	<.01
(g) % glucose uptake undergoing glycolysis	58 ± 3	69 ± 4	<.02
(h) Total glycolytic flux (f × g)	52.6 ± 3.6	66.3 ± 5.5	<.01
(i) Glycogen synthesis (f − h)	37.6 ± 1.5	29.6 ± 3.4	>.05
(j) Carbohydrate oxidation	42.4 ± 3.5	41.7 ± 2.3	>.05
(k) Nonoxidative glucose disposal (b − j)	29.6 ± 2.9	34.3 ± 1.7	>.05

NOTE. Data are expressed in g/5 h, except for (a) in g and (g) in %. Glycogen synthesis (i) is calculated from isotopic data and corresponds to the total amount of glycogen synthesized by the direct pathway. Carbohydrate oxidation (j) and nonoxidative glucose disposal (k) are derived from calorimetry data. Therefore, nonoxidative glucose disposal corresponds to whole-body net carbohydrate balance.

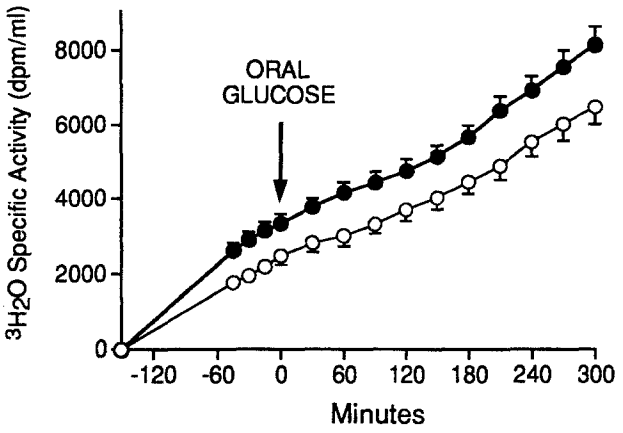

Fig 3. Time course of ^3H_2O content of plasma water during a primed, constant infusion of 3-^{3}H-glucose started at −150 minutes in NIDDM patients (N = 9) treated with either placebo (○) or M (●). Data are normalized for an infusion rate of 1 × 10^6 dpm/min. An oral glucose load was given at 0 minutes.

state and after an oral glucose load in the absence of changes in body weight.

Fasting plasma glucose level was reduced by approximately 20% after M treatment. Although hepatic glucose output was not significantly decreased by the drug, the decrease in glucose concentration correlated positively with the decrease in production ($r = .83$, $P < .01$). In agreement with other reports,[5,10] this observation strongly suggests that a reduction in hepatic glucose output plays an important role in the hypoglycemic effect of the drug. The participation of an increased peripheral extraction of glucose in the hypoglycemic action is suggested by the increase in the MCR, but it is possible that the latter effect is simply the consequence of the decrease in glucose concentration rather than the result of a direct effect of M. It is indeed well established that at constant insulin levels, the MCR is inversely correlated with the glucose concentration, and it has been shown[27] that at the insulin levels in this study (~120 pmol/L), the observed decrease in glucose levels could account for the approximately 15% increase in the MCR recorded in the present experiments.

As shown in other studies,[4,11,28] postprandial glycemia was also systematically reduced after M. The small nonsignificant increase in postprandial insulin levels cannot be ascribed to a direct effect of M, since this drug is devoid of any insulinotropic action.[1] The improvement in insulin secretion is therefore likely to result from the removal of a glucotoxic effect on β cells in relation to the prolonged decrease of glucose levels in the patients.

The rate of entry of oral glucose into the systemic circulation was unaffected by treatment, thus indicating that the hypoglycemic effect of the drug was not related to a reduction of intestinal glucose absorption or to a stimulation of first-pass splanchnic glucose uptake (Fig 1). Since total glucose (exogenous + endogenous) delivered into the systemic circulation was slightly increased after M (85 *v* 80 g/5 h, $P < .05$), the lower postprandial hyperglycemia was entirely accounted for by the stimulation of tissular Rd (85 *v* 78 g/5 h, $P < .001$) in relation to an approximately 33% increase in the MCR (Fig 1). It is possible that this latter effect results from the slight increase in insulin levels, but a direct effect of M cannot be ruled out, inasmuch as the MCR was found to be stimulated to a similar extent in a comparable study in which insulin levels were not affected by the drug.[4]

Besides glucose kinetics, the experimental design used aimed to quantify the effects of M on the pathways of glucose utilization. Some methodological aspects ought to be briefly discussed.

The rate of total glycolysis was estimated from the rate of detritiation of 3-^{3}H-glucose. The use of this method under in vivo conditions was initially proposed by Rossetti et al,[29,30] who showed that in rats[29] and humans[30] the rates of whole-body glycolysis and muscle glycogen synthesis could be accurately determined by this technique during insulin-clamp studies. Under clamp conditions, glucose specific activity and glucose disposal are reasonably steady, ^3H_2O concentration increases in a linear fashion indicating a constant rate of ^3H_2O production, and the rate of glycolysis can be calculated by dividing the ^3H_2O production rate by ^{3}H-glucose specific activity.[30] Under the non–steady-state conditions prevailing in this study, ^{3}H-glucose specific activity and glucose disposal vary continuously with time and the increase in ^3H_2O concentration is not strictly linear (Fig 3). To circumvent this problem, we calculated ^3H_2O production over the 5-hour OGTT period from the difference in ^3H_2O concentration between 300 minutes and 0 minutes multiplied by total-body water volume with urinary elimination of ^3H_2O added. The experiments with 2-^{3}H-glucose indicate that this mode of calculation is probably correct, since approximately 97% of 2-^{3}H-glucose was recovered as ^3H_2O within the time limits of the experiments. Another difficulty arises from the fact that during an OGTT, ^{3}H-glucose specific activity is not only variable with time but also heterogeneous, being probably lower in the portal vein, where intestinal glucose arrives, than in the peripheral circulation, where it is actually measured. Therefore, estimation of the glycolytic rate was based on the calculation of a 0- to 5-hour fractional conversion of 3-^{3}H-glucose to ^3H_2O, multiplied by glucose uptake. Whole-body glucose uptake (which includes splanchnic and peripheral tissues) must obviously be used in this calculation. Even if some inaccuracy is to be expected regarding absolute rates of glycolysis due to the non–steady-state conditions prevailing during an OGTT, the comparison between placebo and M in the same patients should be valid. Thus, under placebo conditions, from the approximately 90 g glucose taken up in 5 hours, approximately 53 g (58%) entered the glycolytic pathway. After M, due to an increase in whole-body glucose uptake (~96 g/5 h) and in the proportion of glucose undergoing glycolysis (69%), glycolytic flux increased to approximately 66 g/5 h, ie, a value about 13 g higher ($P < .01$) than in the absence of the drug. This observation could account for the hyperlactatemic and hyperalaninemic effect of M (Fig 2). Conversely, glycogen formation was reduced (~30 *v* 38 g/5 h), but the difference did not reach statistical significance ($P = .07$). Because glycogen synthesis was calculated as the difference between whole-body glucose uptake and whole-body glycolysis, the figure obtained includes all glycogen formed by the direct pathway in both liver and muscle. Since neither total carbohydrate oxidation measured by indirect calorimetry (Table 2) nor circulating glucose oxidation derived from $^{14}CO_2$ data were affected by the drug, the excess lactate and alanine produced must have entered a nonoxidative pathway, presumably hepatic gluconeogenesis and glycogen-

esis, except for the small unmetabolized amount still present in the body at the end of the study (~1 g). It should be noted in this regard that M has been shown to increase hepatic glycogen content in rats.[31] Thus, during an OGTT, M influences the two pathways of glycogen synthesis in opposite directions, with a decrease of approximately 8 g/5 h in the direct pathway and an increase of approximately 13 g/5 h in the indirect pathway, so that the net effect on whole-body glycogen formation should be a slight increase. The latter conclusion is further supported by the indirect calorimetry data showing that nonoxidative glucose disposal also tends to be increased by M (Table 2). Nonoxidative glucose disposal calculated as the difference between oral glucose uptake and total carbohydrate oxidation represents whole-body net carbohydrate balance, which takes into account glycogen formed by both the direct and indirect pathways. It has been shown previously in hyperinsulinemic-euglycemic clamp studies on obese NIDDM patients that M stimulates nonoxidative glucose metabolism without enhancing insulin activation of muscle glycogen synthase.[10] It is therefore likely that in these studies, as in ours, the increased glycogen formation occurred in the liver rather than in the muscle.

The methodology used in the present study does not permit identification of the tissues responsible for the observed increase in lactate production and glucose uptake, but some speculations are warranted on the basis of the available literature on M action.

Since the hyperlactatemia following glucose ingestion in both nondiabetic[32,33] and diabetic[4] subjects is mainly of splanchnic origin, it could be hypothesized that the extra lactate formed after M originates from that area. Studies by Jackson et al[4] suggest that this is indeed the case, because they observed that M induces an increase rather than a decrease in net forearm lactate uptake after oral glucose. In addition, another antidiabetic biguanide, buformin, has been shown to increase net splanchnic lactate production during an OGTT in normal subjects studied with hepatic vein catheterization,[34] but it is not known whether this applies also to M. On the basis of studies performed in rats,[6,7] the intestine seems to be particularly important as a source of the extra lactate produced after M, because this tissue could account for most of the increased glucose uptake and lactate production in glucose-infused animals, but it must be noted that these effects were observed with high doses of the drug. If the intestine also represents in humans a significant source of extra lactate after M, it could be expected, as discussed earlier, that most of the lactate would be taken up by the liver before being delivered into the systemic circulation, thereby minimizing the hyperlactatemic effect of the drug. It is possible that the observed increase in glucose disposal after M in the present study occurs also in the splanchnic area, but in that case, the increased uptake should necessarily originate from peripheral blood, because the Rd includes only glucose that has been delivered into the systemic circulation.

On the other hand, several arguments can also be put forward in favor of peripheral tissues being the main site of the increase in both glucose uptake and lactate production.[4] With a few exceptions,[11,35] stimulation of peripheral uptake has also been documented after M in euglycemic-hyperinsulinemic[8-10,36,37] and hyperglycemic[11] clamp studies performed in NIDDM patients, and in vitro data on insulin-resistant human skeletal muscle cells have shown that M enhances insulin-stimulated glucose transport.[38] The fact that M stimulates net forearm lactate uptake during an OGTT[4] does not exclude the possibility of an increased nonoxidative glycolysis in peripheral tissues. Indeed, the augmented net lactate uptake might be the result of a stimulation of lactate production by muscle (detected as nonoxidative glycolysis by ^3H_2O production) coexisting with an even greater increase in lactate uptake by other peripheral tissues or even by muscle itself, since it has been shown[39] that the two processes coexist during hyperinsulinemia.

Finally, considering that our data pertain to whole-body metabolism, it is conceivable that the increases in tissular Rd and in lactate production are two independent processes taking place at different sites.

In conclusion, a 3-week treatment of NIDDM patients with M reduces basal hyperglycemia by inhibiting hepatic glucose output and postprandial hyperglycemia by enhancing the uptake and MCR of glucose. These effects can be viewed as an improvement in the overall diabetic status of the patients. On the other hand, M does not correct the abnormalities in the fate of glucose taken up during an OGTT, since it stimulates nonoxidative glycolysis and fails to enhance muscle glycogen synthesis, which has been shown to be deficient in these patients.[40,41] It should be mentioned that these data concern the immediate postprandial period only, and do not permit evaluation of the effects of M on integrated carbohydrate fluxes or on mean glycogen stores in the liver and muscle over a 24-hour period with several successive meals.

ACKNOWLEDGMENT

Thanks are due to M.A. Neef for expert technical assistance. Excellent secretarial aid was provided by C. Demesmaeker.

REFERENCES

1. Bailey CJ: Biguanides and NIDDM. Diabetes Care 15:755-772, 1992

2. Bergman U, Boman G, Wiholm BE: Epidemiology of adverse drug reactions to phenformin and metformin. Br Med J 2:464-466, 1978

3. Vigneri R, Goldfine ID: Role of metformin in treatment of diabetes mellitus. Diabetes Care 10:118-122, 1987

4. Jackson RA, Hawa MI, Jaspan JB, et al: Mechanism of metformin action in non–insulin-dependent diabetes. Diabetes 36:632-640, 1987

5. Stumvoll M, Nurjhan N, Perriello G, et al: Metabolic effects of metformin in non–insulin-dependent diabetes mellitus. N Engl J Med 333:550-554, 1995

6. Bailey CJ, Wilcock C, Day C: Effect of metformin on glucose metabolism in the splanchnic bed. Br J Pharmacol 105:1009-1013, 1992

7. Penicaud L, Hitier Y, Ferre P, et al: Hypoglycaemic effect of metformin in genetically obese (fa/fa) rats results from an increased utilization of blood glucose by intestine. Biochem J 262:881-885, 1989

8. Hother-Nielsen O, Schmitz O, Andersen PH, et al: Metformin improves peripheral but not hepatic insulin action in obese patients with type II diabetes. Acta Endocrinol (Copenh) 120:257-265, 1989

9. McIntyre HD, Bird DM, Paterson CA, et al: Metformin increases insulin sensitivity and basal glucose clearance in type 2 (non–insulin-dependent) diabetes mellitus. Aust NZ J Med 21:714-719, 1991

10. Johnson AB, Webster JM, Sum CF, et al: The impact of metformin therapy on hepatic glucose production and skeletal muscle glycogen synthase activity in overweight type II diabetic patients. Metabolism 42:1217-1222, 1993

11. DeFronzo RA, Barzilai N, Simonson DC: Mechanism of metformin action in obese and lean noninsulin-dependent diabetic subjects. J Clin Endocrinol Metab 73:1294-1301, 1991

12. Widén EIM, Eriksson JG, Groop LC: Metformin normalizes nonoxidative glucose metabolism in insulin-resistant normoglycemic first-degree relatives of patients with NIDDM. Diabetes 41:354-358, 1992

13. Féry F, Balasse EO: Glucose metabolism during the starved-to-fed transition in obese patients with NIDDM. Diabetes 43:1418-1425, 1994

14. Golay A, Felber JP, Jéquier E, et al: Metabolic basis of obesity and noninsulin-dependent diabetes mellitus. Diabetes Metab Rev 4:727-747, 1988

15. Eriksson J, Franssila-Kalunki A, Ekstrand A, et al: Early metabolic defects in persons at increased risk for non–insulin-dependent diabetes mellitus. N Engl J Med 321:337-343, 1989

16. Féry F, D'Attellis NP, Balasse EO: Mechanisms of starvation diabetes: Study with double tracer and indirect calorimetry. Am J Physiol 259:E770-E777, 1990

17. Del Prato S, Bonadonna RC, Bonora E, et al: Characterization of cellular defects of insulin action in type 2 (non–insulin-dependent) diabetes mellitus. J Clin Invest 91:484-494, 1993

18. Féry F, Melot C, Balasse EO: Glucose fluxes and oxidation after an oral glucose load in patients with non–insulin-dependent diabetes mellitus of variable severity. Metabolism 42:522-530, 1993

19. Bergmeyer HU: Methods of Enzymatic Analysis (ed 2). New York, NY, Academic, 1974

20. Morgan CR, Lazarow A: Immunoassay of insulin: Two antibody systems. Plasma insulin levels of normal subdiabetic and diabetic rats. Diabetes 12:115-126, 1963

21. Faloona GR, Unger RH: Glucagon, in Jaffe BM, Behrman HR (eds): Methods of Hormone Radioimmunoassay. New York, NY, Academic, 1974, pp 317-330

22. Steele R: Influence of glucose loading and of injected insulin on hepatic glucose output. Ann NY Acad Sci 82:420-430, 1959

23. Radziuk J, MacDonald T, Rubenstein D, et al: Initial splanchnic extraction of ingested glucose in normal man. Metabolism 27:657-669, 1978

24. Ferrannini E, Bjorkman O, Reichard GA Jr, et al: The disposal of an oral glucose load in healthy subjects. A quantitative study. Diabetes 34:580-588, 1985

25. Ferrannini E: The theoretical basis of indirect calorimetry: A review. Metabolism 37:287-301, 1988

26. Tappy L, Owen OE, Boden G: Effect of hyperinsulinemia on urea pool size and substrate oxidation rates. Diabetes 37:1212-1216, 1988

27. Gottesman I, Mandarino L, Gerich J: Use of glucose uptake and glucose clearance for the evaluation of insulin action in vivo. Diabetes 33:184-191, 1984

28. Hollenbeck CB, Johnston P, Varastch BB, et al: Effects of metformin on glucose, insulin and lipid metabolism in patients with mild hypertriglyceridemia and non–insulin-dependent diabetes by glucose tolerance test criteria. Diabete Metab 17:483-489, 1991

29. Rossetti L, Giaccari A: Contribution of glycogen synthesis and glycolysis to insulin-mediated glucose uptake. A dose-response euglycemic clamp study in normal and diabetic rats. J Clin Invest 84:892-899, 1989

30. Rossetti L, Lee YT, Ruiz J, et al: Quantification of glycolysis and skeletal muscle glycogen synthesis in humans. Am J Physiol 265:E761-E769, 1993

31. Rossetti L, DeFronzo RA, Gherzi R, et al: Effect of metformin treatment on insulin action in diabetic rats: In vivo and in vitro correlations. Metabolism 39:425-435, 1990

32. Jackson RA, Roshania RD, Hawa MI, et al: Impact of glucose ingestion on hepatic and peripheral glucose metabolism in man: An analysis based on simultaneous use of the forearm and double isotope techniques. J Clin Endocrinol Metab 63:541-549, 1986

33. Radziuk J, Inculet R: The effects of ingested and intravenous glucose on forearm uptake of glucose and glucogenic substrate in normal man. Diabetes 32:977-981, 1983

34. Bratusch-Marrain PR, Korn A, Waldhäusl WK, et al: Effect of buformin on splanchnic carbohydrate and substrate metabolism in healthy man. Metabolism 30:946-952, 1981

35. Wu MS, Johnston P, Sheu WHH, et al: Effect of metformin on carbohydrate and lipoprotein metabolism in NIDDM patients. Diabetes Care 13:1-8, 1990

36. Prager R, Schernthaner G, Graf H: Effect of metformin on peripheral insulin sensitivity in non–insulin-dependent diabetes mellitus. Diabete Metab 12:346-350, 1986

37. Nosadini R, Avogaro A, Travisan R, et al: Effect of metformin on insulin-stimulated glucose turnover and insulin binding to receptors in type II diabetes. Diabetes Care 10:62-67, 1987

38. Galuska D, Zierath J, Thorne A, et al: Metformin increases insulin-stimulated glucose transport in insulin-resistant human skeletal muscle. Diabete Metab 17:159-163, 1991

39. Consoli A, Nurjhan N, Gerich JE, et al: Skeletal muscle is a major site of lactate uptake and release during hyperinsulinemia. Metabolism 41:176-179, 1992

40. Mitrakou A, Kelley D, Veneman T, et al: Contribution of abnormal muscle and liver glucose metabolism to postprandial hyperglycemia in NIDDM. Diabetes 39:1381-1390, 1990

41. Kelley D, Mokan M, Veneman T: Impaired postprandial glucose utilization in non–insulin-dependent diabetes mellitus. Metabolism 43:1549-1557, 1994