

METABOLIC EFFECTS OF METFORMIN IN NON-INSULIN-DEPENDENT DIABETES MELLITUS

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Abstract *Background.* The metabolic effects and mechanism of action of metformin are still poorly understood, despite the fact that it has been used to treat patients with non-insulin-dependent diabetes mellitus (NIDDM) for more than 30 years.

Methods. In 10 obese patients with NIDDM, we used a combination of isotope dilution, indirect calorimetry, bioimpedance, and tissue-balance techniques to assess the effects of metformin on systemic lactate, glucose, and free-fatty-acid turnover; lactate oxidation and the conversion of lactate to glucose; skeletal-muscle glucose and lactate metabolism; body composition; and energy expenditure before and after four months of treatment.

Results. Metformin treatment decreased the mean (\pm SD) glycosylated hemoglobin value from 13.2 ± 2.2 percent to 10.5 ± 1.6 percent ($P<0.001$) and reduced fasting plasma glucose concentrations from 220 ± 41 to 155 ± 28 mg per deciliter (12.2 ± 0.7 to 8.6 ± 0.5 mmol per liter) ($P<0.001$). Although resting energy expenditure did

not change, the patients lost 2.7 ± 1.3 kg of weight ($P<0.001$), 88 percent of which was adipose tissue. The mean (\pm SE) rate of plasma glucose turnover (hepatic glucose output and systemic glucose disposal) decreased from 2.8 ± 0.2 to 2.0 ± 0.2 mg per kilogram of body weight per minute (15.3 ± 0.9 to 10.8 ± 0.9 μ mol per kilogram per minute) ($P<0.001$), as a result of a decrease in hepatic glucose output; systemic glucose clearance did not change. The rate of conversion of lactate to glucose (gluconeogenesis) decreased by 37 percent ($P<0.001$), whereas lactate oxidation increased by 25 percent ($P<0.001$). There were no changes in the plasma lactate concentration, plasma lactate turnover, muscle lactate release, plasma free-fatty-acid turnover, or uptake of glucose by muscle.

Conclusions. Metformin acts primarily by decreasing hepatic glucose output, largely by inhibiting gluconeogenesis. It also seems to induce weight loss, preferentially involving adipose tissue. (*N Engl J Med* 1995;333:550-4.)

THE metabolic abnormalities of non-insulin-dependent diabetes mellitus (NIDDM) are generally acknowledged to result from a combination of insulin resistance and impaired insulin secretion.¹ Since 1975, when the biguanide phenformin was withdrawn from the market,² the only drugs available to treat NIDDM orally in the United States have been sulfonylureas, which act primarily by improving insulin secretion.³

Another biguanide, metformin, has recently been approved by the Food and Drug Administration. Although metformin is as effective as the sulfonylureas,⁴ the drugs differ in several respects: metformin reduces insulin resistance without directly affecting insulin secretion,^{4,5} causes weight loss rather than weight gain,⁴ and has lactic acidosis rather than hypoglycemia as its most serious side effect.⁴

Despite the fact that metformin has been in use for more than 30 years, little is known about its primary mode of action or its effects on body composition, energy balance, and lactate metabolism.^{4,5} The rate of entry of lactate into plasma and the conversion of lactate to glucose are both increased in patients with NIDDM.⁶ Metformin reduces hepatic glucose output in NIDDM.⁷⁻¹⁰ In vitro studies suggest that this may be due to the inhibition of gluconeogenesis.^{11,12} Such a mechanism of action might diminish the removal of lactate from plas-

ma and present a risk of lactic acidosis. Phenformin, which was withdrawn from the market because of a high incidence of lactic acidosis,² impaired lactate disposal and increased its production.^{13,14} This study was therefore undertaken to determine the primary mechanism by which metformin improves glycemic control in patients with NIDDM and its effects on lactate metabolism, body composition, and energy expenditure.

METHODS

Subjects

We studied 10 otherwise healthy obese patients with NIDDM (6 men and 4 women). Their mean (\pm SD) age was 58 ± 9 years, and their body-mass index (the weight in kilograms divided by the square of the height in meters) was 32.1 ± 3.2 ; their weight had been stable for several months. The mean (\pm SD) duration of diabetes was 6 ± 3 years. Two patients were being treated with diet, and eight were being treated with sulfonylurea drugs, which were discontinued two weeks before the study. Two patients were taking levothyroxine, two estrogen, one a thiazide, and two an angiotensin-converting-enzyme inhibitor; these drugs were continued at a constant dose throughout the study. The protocol was approved by the Scripps Clinic Institutional Review Board, and all the patients gave written informed consent.

Study Design

The patients underwent the metabolic studies described below before and at the end of a 16-week period of metformin treatment. They were advised to maintain their usual diet and activities during the study. The initial dose of metformin was 850 mg once daily. It was increased to 1700 mg (850 mg twice daily) and then to 2550 mg (850 mg three times daily) at two-week intervals, unless a patient had a fasting plasma glucose concentration below 140 mg per deciliter (7.8 mmol per liter), but that did not happen. During the final 12 weeks, the patients were seen in the clinic every 4 weeks for a pill count and evaluation.

Metabolic Studies

The patients were admitted to the General Clinical Research Center on the evening before the experiments, having consumed a

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weight-maintaining diet containing approximately 200 g of carbohydrate and having abstained from alcohol for at least three days. At about 6 p.m., they ate a standard dinner (10 kcal per kilogram of body weight, 50 percent carbohydrate, 35 percent fat, and 15 percent protein). The next morning, at approximately 6 a.m., primed continuous intravenous infusions of [^{14}C]lactate (30 μCi , 0.3 μCi per minute) and [$6\text{-}^3\text{H}$]glucose (30 μCi , 0.3 μCi per minute) were started and a bolus dose of [^{14}C]sodium bicarbonate (50 μCi) was administered intravenously (all from Amersham International, Little Chalfont, United Kingdom). About 2.5 hours later, an ipsilateral dorsal hand vein was cannulated retrogradely and placed in a thermoregulated device (65°C) for sampling arterialized venous blood. At approximately the same time, a deep vein in the contralateral arm was cannulated retrogradely for sampling venous blood draining muscle tissue. An hour later, a primed continuous intravenous infusion of [$9,10\text{-}^3\text{H}$]palmitate (0.3 μCi per minute, Amersham International) was started. At approximately 10 a.m. and every 40 minutes thereafter for 2 hours, blood was drawn simultaneously from the deep vein and the arterialized hand vein for determinations of plasma substrate and hormone concentrations and of the specific activities of glucose, lactate and palmitate. During this 2-hour interval, total carbon dioxide production and oxygen consumption were measured three times for 30-minute periods with a metabolic monitor (Sensormedics, Anaheim, Calif.) and breath samples were collected for the determination of the specific activity of [^{14}C]carbon dioxide. Forearm blood flow was determined immediately before the first and after the last blood sampling with electrocapacitance plethysmography.¹⁵

Analytic Procedures

The samples from both the pretreatment and post-treatment studies were analyzed at the same time. Plasma glucose concentrations were measured with a glucose analyzer (Yellow Springs Instrument, Yellow Springs, Ohio), plasma lactate by a fluorometric method,¹⁵ and plasma free fatty acid by an enzymatic method.¹⁶ The specific activities of plasma [^3H]glucose, [^{14}C]glucose, and [^{14}C]lactate were measured after lactate and glucose had been isolated with ion-exchange chromatography.¹⁷ The concentration and specific activity of plasma palmitate were determined by high-performance liquid chromatography.¹⁸ Since palmitate represents 30 percent of total plasma free fatty acids, the palmitate results were extrapolated to represent the total plasma free-fatty-acid concentration by dividing the values by 0.30. Total-body muscle mass was calculated from anthropometric measurements according to the equations of Heymsfield et al.¹⁹ Carbohydrate oxidation, lipid oxidation, and resting energy expenditure were measured by indirect calorimetry.²⁰ Body composition (fat mass and fat-free mass) was determined by bioelectrical impedance (RJL Systems, Mt. Clemens, Mich.).²¹

Calculations

The turnover of plasma glucose, lactate, and free fatty acids was calculated with steady-state equations.²² The percentage of glucose derived from lactate was calculated as the specific activity of [^{14}C]glucose \div ($2 \times$ specific activity of [^{14}C]lactate). Glucose turnover from lactate was calculated by multiplying plasma glucose turnover by the percentage of plasma glucose turnover derived from lactate. Plasma lactate oxidation was calculated as ($\text{VCO}_2 \times$ specific activity of [^{14}C]carbon dioxide) \div (specific activity of [^{14}C]lactate $\times 0.81$), where VCO_2 is carbon dioxide production and the factor 0.81 is introduced to correct for the retention of [^{14}C]carbon dioxide in the bicarbonate pool.⁶ The clearance of plasma glucose and lactate was calculated as the turnover rate of each divided by the arterial concentration. The uptake of glucose by the forearm was determined by multiplying the arteriovenous dif-

ference in the glucose concentration by the forearm blood flow (expressed as milliliters per 100 ml of tissue per minute). Forearm net balance, fractional extraction, and the uptake and release of lactate were calculated with standard formulas.²³ Values per 100 ml of forearm tissue were converted to values per kilogram of forearm muscle as previously described²³ and were then extrapolated to represent whole-body muscle by multiplying the values by total-body muscle mass. The clearance of muscle glucose and lactate was calculated by dividing the uptake of each by the arterial concentration.

Statistical Analysis

Unless stated otherwise, the results are expressed as means \pm SE. Plasma glucose, lactate, and free-fatty-acid results represent the mean of four samplings at metabolic steady state. Data before and after treatment with metformin were compared by two-tailed Student's t-test for paired samples and by least-squares regression analysis with a commercially available software package (CSS Statistica, Tulsa, Okla.).

RESULTS

Dosage and Symptoms

All patients received 2550 mg of metformin daily for the last 12 weeks of the 16-week treatment period. Compliance, as determined by regular pill counts, exceeded 95 percent. Most patients initially had some slight abdominal discomfort, bloating, and altered sense of taste lasting one to four weeks. All acknowledged having a mild persistent decrease in appetite.

Weight Loss, Body Composition, and Energy Expenditure

The patients lost approximately 3 kg of weight (Table 1) despite the absence of change in resting energy expenditure or self-reported physical activity. A decrease in body fat mass accounted for about 88 percent of the weight loss, whereas lean body mass did not change.

Glycemic Control and Systemic Glucose Metabolism

Both fasting plasma glucose concentrations and glycosylated hemoglobin values decreased substantially, as

Table 1. Effect of Metformin on Body Composition, Energy Expenditure, and Glycemic Control in Patients with NIDDM.

CHARACTERISTIC	BEFORE TREATMENT	AFTER TREATMENT	NET CHANGE	P VALUE
		<i>mean \pm SD</i>		
Weight (kg)	95.1 \pm 14.9	92.4 \pm 14.5	-2.7 \pm 1.3	<0.001
Lean body mass (kg)	63.8 \pm 11.1	63.5 \pm 10.7	-0.3 \pm 2.2	0.67
Body fat mass (kg)	31.3 \pm 11.4	28.9 \pm 10.1	-2.4 \pm 2.2	0.02
Resting energy expenditure (kcal/min)	1.34 \pm 0.19	1.31 \pm 0.19	-0.03 \pm 0.06	0.20
Glycosylated hemoglobin (%)*	13.2 \pm 2.2	10.5 \pm 1.6	-2.7 \pm 1.6	<0.001
Fasting plasma glucose concentration (mg/dl)†	220 \pm 41	155 \pm 28	-65 \pm 28	<0.001
Fasting plasma glucagon concentration (pg/ml)	108 \pm 21	112 \pm 13	+4 \pm 4	0.29
Fasting plasma insulin concentration ($\mu\text{U/ml}$)‡	12 \pm 5	10 \pm 3	-2 \pm 2	0.04
Fasting plasma lactate concentration (mmol/liter)	1.06 \pm 0.32	0.99 \pm 0.28	-0.07 \pm 0.32	0.54

*Normal range, 4.4 to 7.7 percent.

†To convert values for plasma glucose to millimoles per liter, multiply by 0.05551.

‡To convert values for plasma insulin to picomoles per liter, multiply by 6.

did plasma insulin concentrations; plasma glucagon concentrations did not change. The rate of plasma glucose turnover (hepatic glucose output and systemic glucose disposal) decreased from 2.8 ± 0.2 to 2.0 ± 0.2 mg per kilogram per minute (15.3 ± 0.9 to 10.8 ± 0.9 μmol per kilogram per minute, $P < 0.001$) (Fig. 1). As shown in Figure 2, hepatic glucose output and fasting plasma glucose concentrations both before and after metformin treatment were highly correlated ($r = 0.76$, $P < 0.001$). Although metformin did not alter the rate of systemic glucose clearance (1.3 ± 0.1 ml per kilogram per minute before treatment vs. 1.3 ± 0.1 ml per kilogram per minute afterward, $P = 0.90$) or carbohydrate oxidation (0.8 ± 0.1 vs. 0.8 ± 0.1 mg per kilogram per minute [4.6 ± 0.3 vs. 4.6 ± 0.3 μmol per kilogram per minute], $P = 0.94$), the proportion of glucose disposal accounted for by oxidation increased significantly, from 32 ± 4 to 45 ± 4 percent ($P < 0.001$).

Muscle Glucose Metabolism

Metformin treatment did not alter forearm blood flow, skeletal-muscle mass, or skeletal-muscle glucose uptake (Table 2). The clearance of glucose by muscle increased, as did the proportion of systemic glucose disposal accounted for by muscle.

Systemic and Muscle Lactate Metabolism

Metformin treatment did not significantly alter the mean fasting plasma lactate concentration (Table 1) or

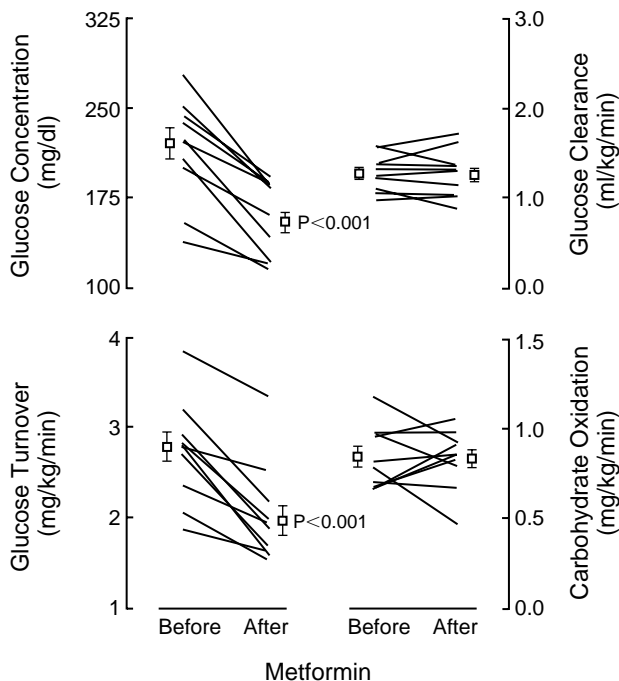


Figure 1. Fasting Plasma Glucose Concentrations, Clearance, and Turnover and Rates of Carbohydrate Oxidation before and after Metformin Treatment in Patients with NIDDM.

To convert values for plasma glucose to millimoles per liter, multiply by 0.05551; to convert oxidation and turnover values to micromoles per kilogram per minute, multiply by 5.551. Means \pm SE are shown, as well as the values for individual subjects.

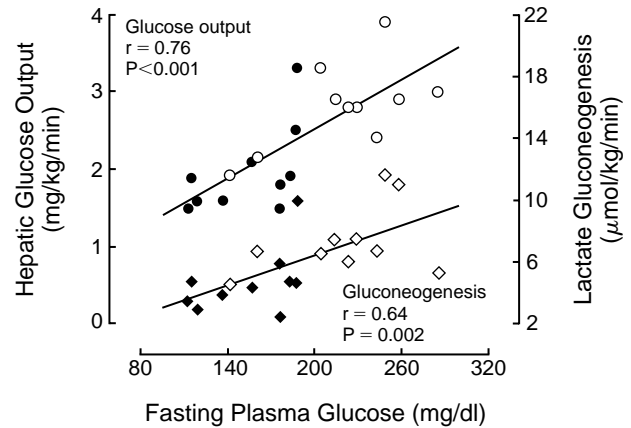


Figure 2. Correlation of Fasting Plasma Glucose Concentrations with Hepatic Glucose Output (Circles) and Lactate Gluconeogenesis (Diamonds) before and after Metformin Treatment in Patients with NIDDM.

Open symbols refer to pretreatment values, and solid symbols to post-treatment values. To convert values for hepatic glucose output to micromoles per kilogram per minute, multiply by 5.551; to convert values for plasma glucose to millimoles per liter, multiply by 0.05551.

the rate of plasma lactate turnover (15.2 ± 1.4 μmol per kilogram per minute before treatment vs. 14.4 ± 1.3 μmol per kilogram per minute afterward, $P = 0.40$) (Fig. 3). However, the rate of plasma lactate oxidation increased by 25 percent (from 6.3 ± 0.6 to 7.9 ± 0.6 μmol per kilogram per minute, $P < 0.001$), whereas the rate of conversion of plasma lactate to plasma glucose decreased by 37 percent (from 7.3 ± 0.7 to 4.6 ± 0.6 μmol per kilogram per minute, $P < 0.001$). Both before and after metformin treatment, the rate of conversion of lactate to glucose and the fasting plasma glucose concentration were highly correlated ($r = 0.64$, $P = 0.002$) (Fig. 2).

Metformin treatment did not alter the clearance, fractional extraction, uptake, or release of lactate by muscle (Table 2).

Plasma Free Fatty Acids and Lipid Metabolism

The mean plasma cholesterol concentration decreased, whereas the mean plasma triglyceride concentration, plasma free-fatty-acid concentration and turnover rate, and whole-body lipid oxidation did not change after metformin treatment (Table 3).

DISCUSSION

In this study, metformin led to an improvement in glycemic control similar to that reported in large, controlled clinical trials.⁴ This improvement was attributable primarily to a reduction in hepatic glucose output, since overall glucose disposal decreased and the rate of systemic glucose clearance did not change. Furthermore, the reduction in hepatic glucose output could be accounted for largely by the inhibition of gluconeogenesis.

After metformin treatment, hepatic glucose output decreased by 0.7 mg (4.5 μmol) per kilogram per min-

Table 2. Effect of Metformin on Skeletal-Muscle Glucose and Lactate Metabolism in Patients with NIDDM.

CHARACTERISTIC	BEFORE TREATMENT	AFTER TREATMENT	NET CHANGE	P VALUE
Forearm blood flow (ml/min/100 ml of tissue)	2.82±0.05	2.78±0.16	-0.04±0.05	0.49
Muscle mass (kg)	28.7±2.4	29.4±2.2	+0.7±0.5	0.13
Muscle glucose metabolism				
Uptake (mg/min)*	24.9±3.1	26.9±2.7	+2.0±3.1	0.56
Clearance (ml/min)	11.8±1.7	18.2±2.6	+6.4±1.7	0.01
Percentage of systemic glucose disposal	10.1±1.6	16.3±2.2	+6.2±1.3	<0.001
Muscle lactate metabolism				
Fractional extraction (%)	18.1±3.8	18.6±1.5	+0.5±3.7	0.90
Clearance (ml/min)	223±63	217±33	-6±59	0.93
Uptake (μmol/min)	249±74	223±45	-26±66	0.72
Release (μmol/min)	482±102	458±70	-24±83	0.79

*To convert values for glucose uptake to micromoles per minute, multiply by 5.551.

ute and the amount of glucose produced from lactate decreased by 0.2 mg (1.4 μmol) per kilogram per minute. Conventional isotopic measurements of the incorporation of lactate into glucose underestimate the amount of lactate converted to glucose by as much as 40 percent,⁶ because of the dilution of radiolabeled carbon in the Krebs cycle. Moreover, the incorporation of lactate into plasma glucose normally accounts for only about 60 percent of overall gluconeogenesis.²⁴ Taking these factors into consideration, we estimate that metformin could have reduced overall gluconeogenesis by as much as 0.6 mg (3.3 μmol) per kilogram per minute. Such a decrease would account for approximately 75 percent of the reduction in hepatic glucose output.

Our results are consistent with those of in vitro studies demonstrating that metformin inhibits hepatic gluconeogenesis^{11,25} and those of clinical studies indicating that most of the increase in hepatic glucose output in patients with NIDDM is due to increased gluconeogenesis.⁶ Our results, however, do not exclude the possibility that metformin may also inhibit glycogenolysis.

Despite the reduced use of lactate for gluconeogenesis during metformin treatment, neither the plasma lactate concentration nor the rate of plasma lactate turnover was changed. Our finding that metformin increases lactate oxidation provides at least a partial explanation for this phenomenon and indicates that metformin differs considerably from phenformin in its effects on lactate metabolism.

Treatment with phenformin increases the plasma lactate concentration and entry of lactate into plasma,²⁶ inhibits lactate oxidation,¹⁴ impairs oxidative phosphorylation,¹³ and increases the release of lactate from muscle.²⁷ Metformin increased lactate oxidation and the proportion of glucose disposal undergoing oxidation while not altering the release of lactate from muscle. These differences may explain why phenformin is associated with a 10-fold to 20-fold greater incidence of lactic acidosis than metformin.⁴ Nevertheless, under conditions impairing the oxidative removal of lactate, the reduced rate of removal of lactate from plasma resulting from decreased conversion of lactate to glucose

by metformin could cause excessive increases in plasma lactate and, possibly, lactic acidosis.

Like previous investigators,^{7,28} we found that energy expenditure did not change during metformin treatment. Since our patients all had a decrease in appetite and denied any change in physical activity, the weight loss accompanying metformin treatment was probably attributable to reduced caloric intake. An unexpected finding was that the weight loss during metformin treatment was largely accounted for by the loss of adipose tissue. In previous studies of body composition in people eating calorically restricted

diets,^{29,30} both fat and lean body mass decreased. For example, in a diet study in which subjects lost amounts of adipose tissue similar to those lost in the present study, the loss of lean body mass averaged 1.5 kg,³⁰ five times more than the small, nonsignificant loss found in the present study.

Differential effects of metformin on adipose tissue and muscle may explain the apparent selective loss of adipose tissue. Whereas the results of in vitro studies³¹ and this study indicate that metformin improves insulin sensitivity in muscle glucose metabolism, it does

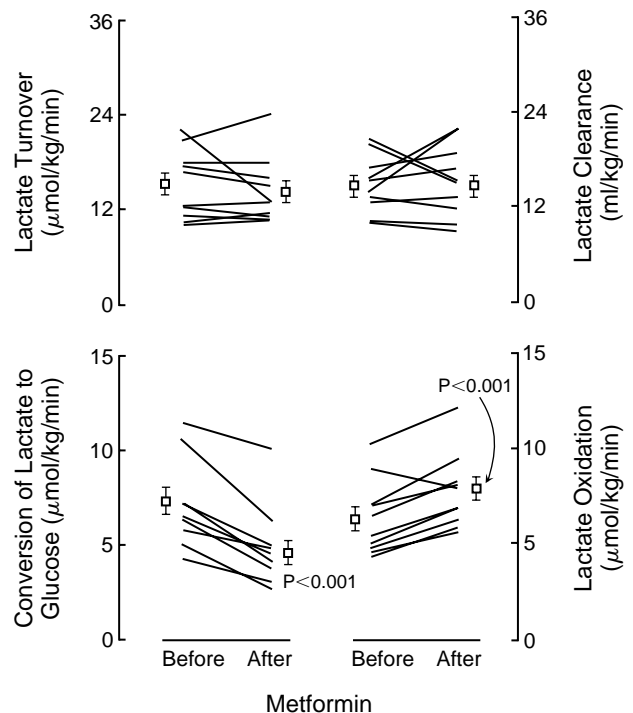


Figure 3. Plasma Lactate Turnover, Clearance, and Oxidation and Rates of Conversion of Lactate to Glucose before and after Metformin Treatment in Patients with NIDDM.

Means ±SE are shown, as well as the values for individual subjects.

Table 3. Effect of Metformin Treatment on Plasma Free Fatty Acids and Lipid Metabolism in Patients with NIDDM.

CHARACTERISTIC	BEFORE TREATMENT	AFTER TREATMENT	NET CHANGE	P VALUE
	<i>mean ±SE</i>			
Plasma cholesterol (mg/dl)*	243±8	210±6	-33±7	0.005
Plasma triglycerides (mg/dl)†	260±47	228±31	-32±20	0.15
Plasma free fatty acids				
Concentration (μmol/liter)	688±39	655±18	-33±44	0.50
Turnover (μmol/kg/min)	8.9±0.8	8.8±1.1	-0.1±0.7	0.96
Whole-body lipid oxidation (mmol/kg/min)	3.5±0.1	3.6±0.1	+0.1±0.1	0.41

*To convert values for cholesterol to millimoles per liter, multiply by 0.0259.

†To convert values for triglycerides to millimoles per liter, multiply by 0.0113.

not affect the antilipolytic action of insulin on adipose tissue.³² Since plasma insulin concentrations decrease during metformin treatment,⁴ one would expect lipolysis to increase. On the other hand, if the metformin-induced increase in the sensitivity of muscle to insulin included an anticatabolic effect of insulin on protein metabolism, one would expect no change in lean tissue mass.

In many^{4,8} but by no means all studies,^{7,9,10} metformin treatment was accompanied by an improvement in insulin-stimulated systemic glucose disposal. We found that the uptake of glucose by muscle did not decrease despite a reduction in plasma glucose concentrations, indicating that the efficiency of muscle glucose uptake was increased. This increase was documented by our finding of an increase in the clearance of glucose by muscle. Since these changes occurred despite the presence of reduced plasma insulin concentrations, it appears that metformin treatment improved the sensitivity of muscle to insulin. However, whether this improvement represents a direct effect on muscle or an indirect effect due to weight loss³³ or a decrease in glucose-induced insulin resistance³⁴ is unclear.

In conclusion, metformin treatment improves glycaemic control and decreases fasting hyperglycemia in patients with NIDDM, primarily by decreasing hepatic glucose output, an effect largely accounted for by the inhibition of gluconeogenesis. Metformin improves the sensitivity of muscle to insulin and the oxidative disposal of glucose and lactate in the whole body, while not altering muscle lactate metabolism, the plasma lactate concentration, or plasma lactate turnover. Finally, weight loss associated with metformin treatment appears to involve a preferential loss of adipose tissue.

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