Effect of Metformin on Insulin-Stimulated Glucose Turnover and Insulin Binding to Receptors in Type II Diabetes

R. NOSADINI, A. AVOGARO, R. TREVISAN, A. VALERIO, P. TESSARI, E. DUNER, A. TIENGO, M. VELUSSI, S. DEL PRATO, S. DE KREUTZENBERG, M. MUGGEO, AND G. CREPALDI

Euglycemic insulin glucose-clamp and insulin-binding studies on erythrocytes and monocytes were performed in seven type II (non-insulin-dependent) diabetic subjects before and after 4 wk of metformin treatment (850 mg 3 times/day) and in five obese subjects with normal glucose tolerance. Glucose turnover was also measured at basal insulin concentrations and during hyperinsulinemic euglycemic clamps. During euglycemic insulin-glucose clamps, diabetic subjects showed glucose disposal rates of 3.44 ± 0.42 and 7.34 ± 0.34 mg · kg^{-1} · min^{-1} (means ± SD) before metformin at insulin infusion rates of 0.80 and 15.37 mU · kg^{-1} · min^{-1}, respectively. With the same insulin infusion rates, glucose disposal was 4.94 ± 0.55 ($P < .01$) and 8.99 ± 0.66 ($P < .01$), respectively, after metformin treatment. Glucose disposal rates in normal obese subjects were 5.76 ± 0.63 ($P < .01$) and 10.92 ± 1.11 ($P < .01$) at 0.80 and 15.37 mU · kg^{-1} · min^{-1}, respectively. Insulin maximum binding to erythrocytes in diabetics was 9.6 ± 4.2 and 5.8 ± 2.6 × 10^9 cells (means ± SD) before and after metformin treatment, respectively (NS). Insulin maximum binding to monocytes in diabetics was 6.2 ± 2.3 × 10^7 cells before and 5.0 ± 1.6% after metformin. Hepatic glucose production was higher in the diabetic patients at basal insulin levels, but not at higher insulin concentrations, and was not significantly changed by drug treatment. Basal glucose and insulin concentrations decreased with metformin. Thus, metformin treatment improved glucose disposal rate without significant effect on insulin-binding capacity on circulating cells. Basal hepatic glucose output was slightly lower after metformin treatment in view of lower (9 vs. 15 μU/ml) insulin levels, potentially indicating increased sensitivity of the liver to insulin. *Diabetes Care* 10:62–67, 1987

Type II diabetic subjects are both insulin deficient and insulin resistant (1–3). Insulin resistance is due to a combined receptor and postreceptor defect, the latter being the predominant lesion (4,5). Thus, treatment of type II diabetes should be aimed at improving the response of tissues to insulin. Both sulfonylureas (6–8) and insulin (9) were found to be useful therapeutic agents when diet alone had failed. Biguanides could also play a useful role in the treatment of type II diabetic patients with normal hepatic, renal, and cardiovascular function. Several years ago, Butterfield and Wichelow (10) suggested that phenphormin stimulates muscle glucose uptake without stimulating insulin secretion. Recently, few reports have suggested that biguanides can increase insulin binding to receptors in erythrocytes from normal subjects (11) and to receptors in cultured lymphocytes and cultured breast cancer cells (12).

We investigated the effects of 4 wk of metformin treatment with the euglycemic insulin glucose-clamp and isotopic techniques to evaluate glucose metabolism in vivo in type II diabetic subjects after unsuccessful treatment with diet. Insulin-binding studies on circulating cells were also performed to determine whether metformin modulates insulin-receptor interaction.

SUBJECTS AND METHODS

Subjects. Five obese subjects with normal oral glucose tolerance and seven type II diabetic patients participated in the study, according to the criteria of the National Diabetes Data Group (13). No subject was taking any drug affecting carbohydrate metabolism. All subjects were placed on an isocaloric diet with three meals daily (50% carbohydrate, 25%

fat, and 25% protein by weight) for at least 30 days before the study. The patients were hospitalized for at least 1 wk before the insulin-glucose clamp study. The group of control subjects was chosen from patients who exhibited normal glucose tolerance admitted to our ward because of obesity. All subjects gave informed consent after thorough explanation of the study procedure. Body weight was stable in the last month before clamp study.

After the first glucose study, the patients were studied on metformin treatment (Glucophage, Spemsa, Florence, Italy; 850 mg 3 times/day for 4 wk). The diet was the same as the previous month. After 4 wk of treatment, a second glucose clamp was performed. No significant changes were observed in body weight before and after drug treatment. Subjects who had >8% weight loss were excluded from the study. Thus, we did not consider the results of 3 of 10 type II diabetic patients, who exhibited a 15–20% weight loss after drug administration.

Glucose-clamp study. A wrist vein was cannulated in a retrograde manner, and the hand was placed in a warm box (60°C). Intermittent blood samples (at 5-min intervals) were drawn for rapid plasma glucose determination (glucose analyzer, Beckman, Fullerton, CA). An artificial endocrine pancreas (Biostator, Life Science Instruments, Miles, FRG) was connected to the patient via a double-lumen catheter placed in an antecubital vein. The continuous glucose reading of the Biostator was calibrated to the external value determined on arterialized blood. The Biostator maintained constant glycemia, infusing glucose (400 g/L) according to the algorithm (14)

$$DR = wt \cdot KS \cdot \left(4BC - \frac{GY}{3 + M}\right) + RC$$

where DR is glucose infusion rate (mg/min); wt is body weight (kg); BC is desired blood glucose value (mg/dl); GY = observed blood glucose; *KS* is constant value; RC is 0.9 previous RC + 0.1 (DR/wt). Further details are given elsewhere (14–19).

All subjects were clamped at euglycemic blood glucose value. Insulin (Actrapid MC, Novo, Copenhagen) diluted in 66 ml saline plus 2 ml of the subject's blood was infused with a syringe pump (Harvard Apparatus, Millis, MA) at constant infusion rates of 0.80 and 15.37 mU · kg^{-1} · min^{-1} from 10 to 150 and from 160 to 300 min. During the 0- to 10- and 150- to 160-min intervals, a priming insulin dose logarithmically decreasing proportional to each of the two constant infusion rates was administered. Because all diabetic subjects showed plasma glucose values >90 mg/dl before and after drug treatment, a preinfusion of insulin (0.20 mU · kg^{-1} · min^{-1}) was administered in both normal and diabetic subjects together with glucose clamp to achieve euglycemia at the beginning of the euglycemic clamp. Euglycemia was achieved in 53 ± 8 and 27 ± 11 min ($P < .05$) in the diabetic obese group before and after drug treatment, respectively. Insulin infusion (0.20 mU · kg^{-1} · min^{-1}) was then commenced in normal subjects and continued in diabetic subjects for 90 min. Variable glucose amounts were infused for 90 min before time 0 of euglycemic clamp (1640 ± 209 and 5982 ± 419 mg before and after metformin treatment, respectively, in diabetic patients and 8229 ± 713 mg in normal subjects).

Intermittent blood sampling was performed every 10 min for insulin measurements (15). Endogenous glucose production before and after insulin infusion was calculated with a constant infusion of D-6-[^{3}H]glucose (sp act 222 mCi/mg, Batch 38; Amersham International Amersham, Bucks, UK) preceded by a priming dose of 20 μCi, at basal insulin concentrations during the 0.20- and 0.80-mU · kg^{-1} · min^{-1} insulin infusion rates.

^{125}I-labeled insulin binding to erythrocytes. Heparinized blood samples (10 ml) were drawn before each clamp study. The cells were diluted 1:1 with phosphate-buffered saline, (pH 7.4), layered onto Ficoll-Hypaque gradients, and centrifuged at 400 × *g* for 20 min (16,20). The mononuclear cells and the granulocytes were aspirated and discarded together with the top layer of erythrocytes (~10% of the original volume of red cells). This step was repeated twice under the same conditions. The red cells were washed in Hepes-Tris buffer (pH 8) and resuspended in the same buffer to a concentration of 4.5 × 10^9 erythrocytes/ml. Contamination with leukocytes was 2 leukocytes/10^6 cells, and no platelets were found in the preparation. The reticulocyte content was 0.1%. Cells (0.4 ml) were incubated (in a total volume of 0.5 ml) with ^{125}I-insulin (0, 1 ng/ml) in the absence and presence of unlabeled insulin over a range of concentrations from 0 to 100 μg/ml for 90 min at 15°C. After the incubation period, 0.2 ml replicate aliquots were transferred to chilled microcentrifuge tubes containing 0.2 ml of assay buffer and 0.2 ml of diisobutylphthalate and centrifuged in a Beckman B microcentrifuge. The supernatant was aspirated and discarded; the cell pellet was excised, and the radioactivity in the pellet was counted. Nonspecific binding was defined as the radioactivity in the cell pellet, in the presence of 100 μg/ml of unlabeled insulin, and was subtracted from each point.

^{125}I-insulin binding to monocytes. Blood (100 ml) was drawn into heparinized tubes and centrifuged. Plasma was aspirated, and the cells were diluted 1:1 with phosphate-buffered saline (pH 7.4) layered onto Ficoll-Hipaque gradients and centrifuged. The mononuclear cell layer (monocytes plus lymphocytes) was removed and diluted in 100 mM Hepes-Tris buffer (pH 8.0) to a final concentration of 5 × 10^7 cells/ml. The monocyte has been shown to be major insulin-binding cell in this preparation (21). The percentage of monocytes in the final preparation was determined by phagocytosis of latex beads, and the results were expressed as binding per 1.0 × 10^7 monocytes/ml. ^{125}I-porcine insulin was prepared at sp act of 120–180 μCi/μg by a modified chloramine-T method and purified by chromatography on a cellulose column. The cells were incubated with ^{125}I-insulin in the absence and presence of unlabeled insulin over a range of concentrations from 0.2 to 10^5 ng/ml, a total volume of 0.5 ml for 180 min at 22°C. Replicate aliquots were transferred to chilled microcentrifuge tubes and centrifuged in a Beckman microcentrifuge. The

supernatant was aspirated and discarded; the cell pellet was excised, and the radioactivity in the pellet was counted. The percentage of total radioactivity specifically bound to receptors is plotted as a function of the total insulin concentrations. Further details are given elsewhere (16,20).

Calculations. The rates of glucose utilization were calculated during only the last 70-min period during insulin infusion (80–150 and 230–300 min). Rates of endogenous glucose production were calculated according to Norwich (22). On occasion, the theoretical value, calculated by subtracting the amount of glucose infused from the rate of glucose appearance calculated with the isotopic technique, was paradoxically negative during the insulin infusion period at the highest rate. In this case, endogenous production was taken as zero. Statistical comparisons were performed with Dunnett's statistical analysis for multiple-group comparison. All data are expressed as means ± SD.

RESULTS

Clinical data. The age, weight, body mass index, and sex characteristics of diabetic and nondiabetic subjects are shown in Table 1. Ideal body weight was not significantly higher in the diabetic than in the obese normal subjects. Metformin treatment did not result in a significant decrease in body weight. Both fasting plasma glucose and insulin levels were higher in diabetic than in normal subjects and were reduced by metformin treatment ($P < .01$ and $P < .01$, respectively; Table 1).

Insulin glucose dose-response curves. The plasma insulin plateaus during the 0.80-mU · kg^{-1} · min^{-1} insulin infusion rate were 89 ± 7, 92 ± 6, and 90 ± 8 μU/ml (means ± SD) in normal and diabetic subjects before and after metformin treatment, respectively (Fig. 1 and Table 2). During the euglycemic clamp at 15.37 mU · kg^{-1} · min^{-1}, insulin plateaus were 1894 ± 102 in normal and 1851 ± 71 and 1863 ± 69 μU/ml in diabetic subjects before and after metformin, respectively. In view of similar insulin concentrations during the 0.80-mU · kg^{-1} · min^{-1} infusion clamp, glucose disposal rate was lower in diabetic patients than in normal subjects (3.44 ± 0.42 vs. 5.76 ± 0.63 mg · kg^{-1} · min^{-1}, respectively). Metformin treatment significantly increased ($P < .01$) the insulin-stimulated glucose disposal rate (4.94 ± 0.55). During the 15.37-mU · kg^{-1} · min^{-1} infusion clamp, the glucose disposal rate was 7.34 ± 0.34 and 8.99 ± 0.66 mg · kg^{-1} · min^{-1} ($P < .01$) in diabetics before and after metformin, respectively, and 10.92 ± 1.11 mg · kg^{-1} · min^{-1} in normal subjects. Table 2 shows plasma glucose and blood insulin plateaus together with their coefficients of variation.

Insulin-receptor binding. ^{125}I-insulin maximum binding to red cells was 6.8 ± 0.6 × 10^9 cells in normal subjects and 9.6 ± 4.2 and 5.8 ± 2.6% × 10^9 cells in diabetic patients before and after metformin treatment, respectively (Fig. 2, *upper panel*). ^{125}I-insulin maximum binding to monocytes was 7.0 ± 1.5 and 6.2 ± 2.3 and 5.0 ± 1.6% × 10^7 cells in normal and diabetic subjects before and after metformin treatment, respectively (Fig. 2, *lower panel*).

Glucose turnover. Basal glucose production was higher in diabetic patients before metformin treatment than in obese normal subjects (2.22 ± 0.17 vs. 1.79 ± 0.18 mg · kg^{-1} · min^{-1}, $P < .05$, means ± SE, respectively). Metformin treatment decreased basal glucose production rate in diabetic subjects, but the difference did not reach statistical significance (2.01 ± 0.21 mg · kg^{-1} · min^{-1}).

DISCUSSION

In the type II patient, in whom obesity and resistance to insulin are present in most cases (4,5,23), the demand for insulin is increased in view of an impaired endogenous capacity to provide adequate amounts of hormone (3). Particular attention has been paid recently to attempts to improve peripheral glucose utilization in type II

TABLE 1
Clinical data on type II patients before and after metformin treatment compared with a group of obese subjects with normal oral glucose tolerance tests

Diabetic obese subjects	Weight		Body mass index		Gender	Age (yr)	Fasting plasma glucose (mg/dl)		Fasting plasma insulin (mU/L)	
	Before treatment	After treatment	Before treatment	After treatment			Before treatment	After treatment	Before treatment	After treatment
1	94	95	29.6	29.9	F	42	122	107	18	12
2	119	118	35.5	35.2	M	49	149	109	21	9
3	90	89	30.4	30.0	F	41	162	111	14	10
4	81	82	28.3	28.6	F	51	157	109	13	8
5	82	83	26.2	26.5	M	54	182	140	14	7
6	84	85	26.2	26.5	M	47	149	101	10	5
7	77	78	25.4	25.7	M	42	171	115	19	12
Means ± SD	89 ± 14	90 ± 13	28.9 ± 3.5	28.9 ± 3.2		46 ± 5	156 ± 19	113 ± 12	15 ± 4	9 ± 3
Normal obese subjects (N = 5)	85 ± 6		27.3 ± 4.2		3M,2F	41 ± 5	80 ± 6		10 ± 4	

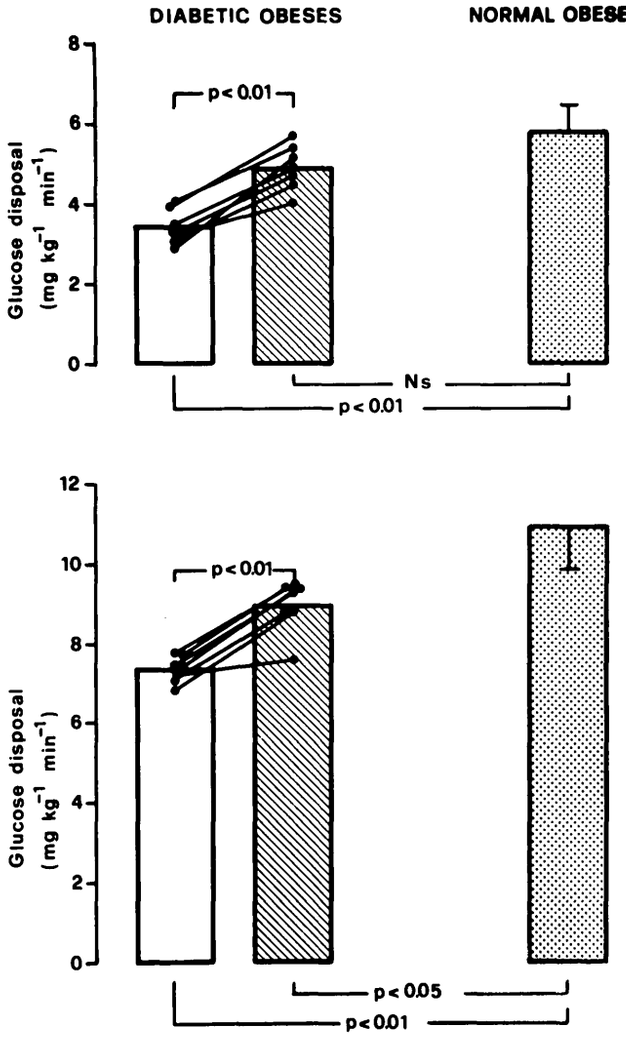

FIG. 1. Glucose disposal rate during euglycemic insulin glucose clamp in diabetic subjects before (□) and after (▨) metformin treatment and in normal obese subjects (⊡). Values expressed as means ± SD. *Upper panel* shows glucose disposal rate during 0.80 mU · kg^{-1} · min^{-1} insulin infusion rate. *Lower panel* shows results of clamp study at 15.37 mU · kg^{-1} · min^{-1} insulin infusion rate.

diabetes with either sulfonylureas (6–8,21) or insulin (9). Biguanides are other pharmacologic agents that may increase peripheral glucose utilization regardless of the presence of insulin (24).

Conflicting results, however, have been obtained on the effects of biguanides on peripheral glucose utilization, which was found to be lessened in healthy people (25), unchanged (24), or increased (10) in type II diabetic patients after drug treatment. Interest in biguanides was recently renewed by reports that metformin increases insulin binding to receptor in erythrocytes of normal subjects after 3 days of treatment (11) and to receptors in cultured lymphocytes and cultured breast cancer cells (12). However, few studies have been performed in type II diabetic patients after >1 wk of treatment to confirm these preliminary reports.

Our results indicate that 4 wk of metformin treatment improves peripheral removal of glucose in type II diabetes, mainly improving insulin action at a postreceptor step because a significant increase of maximal glucose disposal rate was observed without any change of insulin binding on circulating cells. The discrepancy between these conclusions and the previous results in normal people obtained by Holle et al. (11) could be related to the differences in study protocol. We have investigated type II diabetics after 4 wk of metformin, whereas the effects of metformin in that study were observed in normal subjects and after only 3 days of metformin treatment. While this study was in progress, Lord et al. (26) reported that metformin treatment increased low-affinity binding sites on erythrocytes by 184% in type II diabetics. Diabetic control, as assessed by urinary glucose, glycosylated hemoglobin (HbA_{1c}), and glucose tolerance values were significantly improved, whereas plasma insulin concentrations were not altered. These authors suggest that their results indicate that the increase in low-affinity insulin receptors is responsible for the greater insulin sensitivity and improved diabetic control. Our data do not seem to support this hypothesis because, although we confirmed that metformin clearly enhances insulin-mediated glucose removal, this effect was not associated with any significant change in insulin-binding capacity, suggesting that metformin treatment influences insulin action at the postreceptor level. The latter finding is further supported by the observation that metformin improves glucose disposal rate also at 2000-μU/ml insulin concentrations, i.e., it is capable of influencing insulin responsiveness and not just insulin sensitivity (14).

TABLE 2
Plasma concentrations of glucose, insulin, and hepatic glucose production during the euglycemic-clamp studies before and after metformin (means ± SD)

	Obese normal subjects	Obese diabetic subjects	
		Before	After
Glucose (mg/dl)			
80–150 min	88 ± 4	90 ± 3	91 ± 4
C.V. (%)	5 ± 1	4 ± 2	6 ± 1
230–300 min	90 ± 5	88 ± 6	89 ± 4
C.V. (%)	4 ± 1	4 ± 1	5 ± 2
Insulin (μU/ml)			
80–150 min	89 ± 7	92 ± 6	90 ± 8
C.V. (%)	9 ± 2	11 ± 3	11 ± 2
230–300 min	1894 ± 102	1851 ± 71	1863 ± 69
C.V. (%)	12 ± 3	14 ± 2	15 ± 3
Hepatic glucose production (mg · kg^{-1} · min^{-1})			
Overnight fasting	1.79 ± 0.18	2.22 ± 0.17*	2.01 ± 0.21
First clamp (80–150 min)	0.07 ± 0.08	0.09 ± 0.06	0.08 ± 0.06

C.V., coefficient of glucose-plateau variation
*$P < .5$

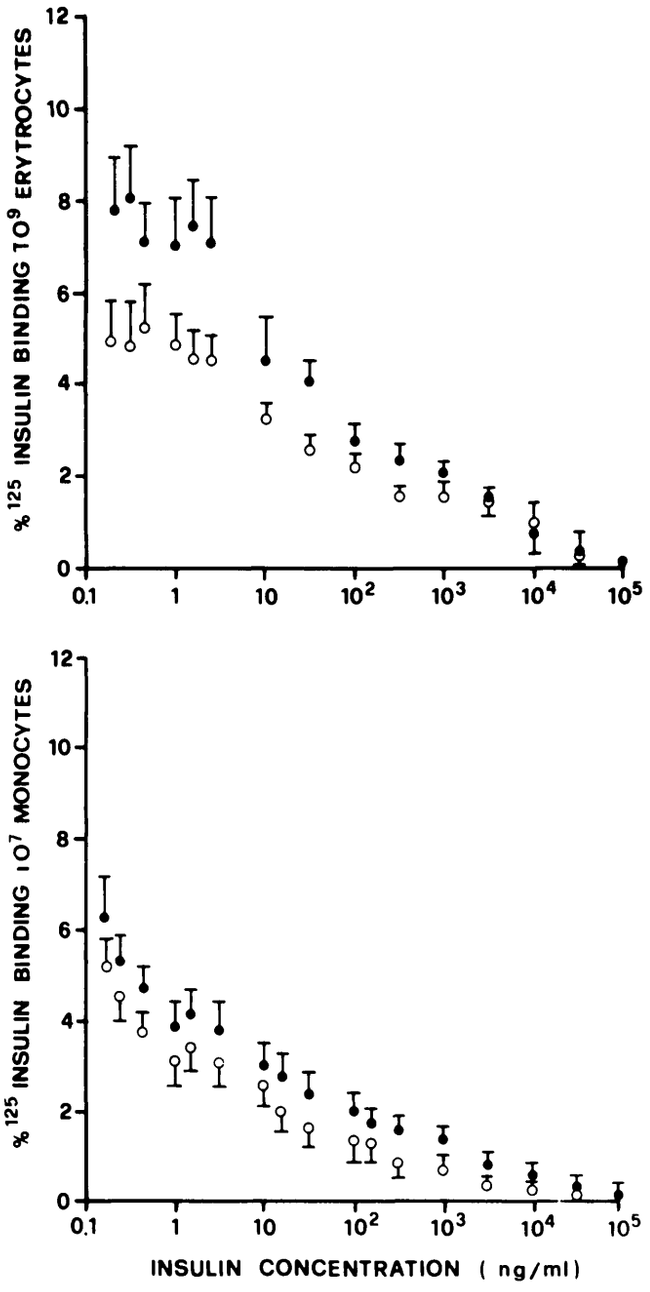

FIG. 2. *Upper panel* shows insulin binding on erythrocytes from diabetic subjects before (●) and after (○) metformin treatment. Results were corrected for nonspecific binding and indicate ^{125}I-insulin specifically bound/10^9 red cells. *Lower panel* shows insulin binding on monocytes from diabetic subjects before (●) and after (○) metformin. Results were corrected for nonspecific binding and indicate ^{125}I-insulin specifically bound/10^7 monocytes (means ± SD).

However, some considerations of Lord et al. (26) seem to support our conclusion that the improvement in glucose metabolism after metformin treatment is poorly related to insulin receptor changes. First, the increase in insulin binding in this report was still present 4 wk after metformin had been withdrawn, although glycemia had returned to almost pretreatment values, suggesting a poor relationship between metabolic events and insulin-receptor changes (26). Second, the same group of researchers showed that metformin, like glyburide, increased insulin sensitivity in diabetic mice without a measurable change in hepatocyte insulin binding (27).

With regard to the hepatic glucose output, diabetic subjects showed significantly elevated rates of glucose production on the liver compared with normal obese subjects, despite the fact that plasma insulin concentrations were significantly elevated. Thus, these findings suggest that resistance to the metabolic effects of insulin characterize not only glucose metabolism in the extrahepatic tissues but also in the livers in type II diabetes, at least at basal insulin levels. However, metformin treatment was capable of improving insulin action mainly in the extrahepatic tissues, because the increase in glucose disposal rate during euglycemic-clamp treatment was not associated with a significant reduction of liver glucose output. With regard to this issue, however, note that the basal hepatic glucose output was slightly, although not significantly, lower after metformin treatment in view of decreased insulin levels (9 vs. 15 μU/ml), indicating increased sensitivity of the liver to insulin effects. Thus, our data do not rule out the possibility that the metabolic action of metformin on extrahepatic tissues is associated with a similar effect of this drug at liver site.

In conclusion, our data indicate that metformin improves insulin-stimulated glucose disposal in type II diabetics in the extrahepatic tissues without an effect on insulin-binding capacity on circulating erythrocytes and monocytes. Further investigation is needed to clarify whether metformin can modulate insulin action mainly in muscle at the postreceptor level. Hepatic glucose production may be better modulated by insulin after metformin administration.

ACKNOWLEDGMENTS: This study was supported by The National Council of Research (Grants 82.02.42304, 82.02.32656, and 83.02857.56).

From the Cattedra di Patologia Medica I (R.N., A.V., P.T., E.D., G.C.) and Cattedra di Malattie del Ricambio, (A.A., R.T., A.T., S.D.P., S.D.K.), Università di Padova, Padova, Monfalcone, USL 3, Centro antidiabetico (M.V.), and Cattedra di Malattie del Ricambio, Università di Verona (M.M.), Verona, Italy.

Address correspondence and reprint requests to Dr. R. Nosadini, Cattedra di Patologia Medica I, Istituto di Medicina Interna, Policlinico, Via Giustiniani, 2, 35100 Padova, Italy.

REFERENCES

1. Ginsberg H, Kimmerling G, Olefsky J, Reaven GM: Demonstration of insulin resistance in untreated adult-onset diabetic subjects with fasting hyperglycaemia. *J Clin Invest* 55:454–61, 1975
2. Davis BM, Bernstein R, Kolterman O, Olefsky JM, Reaven GM: Defect in glucose removal in nonketotic diabetic patients with fasting hyperglycemia. *Diabetes* 28:32–34, 1979

3. Rizza RA, Mandarino J, Gerich JE: Mechanism and significance of insulin resistance in non-insulin-dependent diabetes mellitus. *Diabetes* 30:990–95, 1981
4. Kolterman O, Gray R, Griffin J, Burnstein P, Insel J, Scarlett J, Olefsky J: Receptor and post-receptor defects contribute to the insulin resistance in non-insulin dependent diabetes mellitus. *J Clin Invest* 68:957–69, 1981
5. Olefsky J, Kolterman O: Mechanisms of insulin resistance in obesity and non-insulin-dependent (type II) diabetes. *Am J Med* 70:151–68, 1981
6. Lebovitz H, Feinglos M: Therapy of insulin independent diabetes mellitus. General considerations. *Metabolism* 29:474–80, 1980
7. Olefsky J, Reaven G: Effects of sulfonylurea therapy on insulin binding to mononuclear leukocytes of diabetic patients. *Am J Med* 60:89–95, 1976
8. Putuam W, Andersen D, Jones R, Lebovitz H: Selective potentation of insulin-mediated glucose disposal in normal dogs by the sulfonylurea glipizide. *J Clin Invest* 67:1016–23, 1981
9. Scarlett JA, Gray RS, Griffin JM, Olefsky JM, Kolterman OG: Insulin treatment reverses the insulin resistance of type II diabetes mellitus. *Diabetes Care* 5:353–63, 1982
10. Butterfield W, Wichelow M: Effect of diet, sulfonylureas and phenphormin on peripheral glucose uptake in diabetes and obesity. *Lancet* 2:785–93, 1968
11. Holle A, Mangels W, Dreyer M, Kuharm J, Rudiger HW: Biguanide treatment increases the number of insulin receptor sites on human erythrocytes. *N Engl J Med* 1305:563–66, 1981
12. Vigneri R, Pezzino V, Wong KY, Golfine ID: Comparison of the in vitro effect of biguanides and sulfonylureas on insulin binding to its receptors in target cells. *J Clin Endocrinol Metab* 54:95–101, 1982
13. National Diabetes Data Group: Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. *Diabetes* 28:1039–53, 1979
14. Clemens AH, Hough DL, D'Orazio PA: Development of the Biostator glucose clamping algorithm. *Clin Chem* 28:1899–908, 1982
15. Nakagawa S, Nakayama H, Sasaki T, Yoshino K, Yune YY, Shinozaki K, Aoki S, Mashimo K: A simple method for the determination of serum free-insulin levels in insulin-treated patients. *Diabetes* 22:590–600, 1973
16. Nosadini R, Del Prato S, Tiengo A, Duner E, Toffolo G, Cobelli C, Faronato PP, Moghetti P, Muggeo M: Insulin sensitivity, binding and kinetics in pancreatogenic and type I diabetes. *Diabetes* 31:346–55, 1982
17. Nosadini R, Del Prato S, Tiengo A, Valerio A, Muggeo M, Opocher G, Mantero F, Duner E, Marescotti MC, Mollo F, Belloni F: Insulin resistance in Cushing's syndrome. *J Clin Endocrinol Metab* 67:529–36, 1983
18. DeFronzo R, Tobin JD, Andres R: Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 237:E214–23, 1979
19. Kahn CR: Insulin resistance, insulin insensitivity and insulin unresponsiveness: a necessary distinction. *Metab Clin Exp* 27 (Suppl. A):1893–902, 1978
20. Wachslicht-Rodbard H, Gross HA, Rodbard D, Ebert MH, Roth J: Increased insulin binding to erythrocytes in anorexia nervosa. *N Engl J Med* 300:882–87, 1979
21. Maloff B, Lockwood D: In vitro effects of a sulfonylurea an insulin action in adipocytes. *J Clin Invest* 68:85–90, 1981
22. Norwich KH: *Dynamics in Biosystem*. Oxford, UK, Pergamon, 1977
23. Reaven GM, Bernstein R, Davis B, Olefsky JM: Nonketotic diabetes mellitus: insulin deficiency or insulin resistance? *Am J Med* 1976; 60:80–89.
24. Madison L, Unger R: Effect on phenphormin on peripheral glucose utilization in human diabetic and nondiabetic subjects. *Diabetes* 9:202–206, 1960
25. Dietze G, Wicklmayr M, Bram S, Hepp KD, Melmert H: Untersuchungen zur Wirking von Biguaniden am Gesunden. Die glukose utilization des Muskels unter dem Einfluss von Phenphormin. *Verh Dtsch Ges Inn Med* 80:1283–86, 1974
26. Lord J, White S, Bailey C, Atkins T, Fletcher R, Taylor K: Effect of metformin on insulin receptor binding and glycaemic control in type II diabetes. *Br Med J* 286:830–31, 1983
27. Lord J, Atkins T, Bailey C: Metformin and glibenclamide effects on hepatocyte insulin receptor binding in normal and streptozotocin diabetic mice (Abstract). *Diabetologia* 21:551, 1981