Effect of Metformin on Glucagon-Like Peptide 1 (GLP-1) and Leptin Levels in Obese Nondiabetic Subjects

EDOARDO MANNUCCI, MD[1]
AGOSTINO OGNIBENE, MD[2]
FRANCESCO CREMASCO, MD[1]
GIANLUCA BARDINI, MD[1]
ANTONELLA MENCUCCI, MD[1]
ENRICA PIERAZZUOLI, MD[1]
SILVIA CIANI, BS[1]
GIANNI MESSERI, MS[2]
CARLO M. ROTELLA, MD[1]

OBJECTIVE — To evaluate the effects of metformin on glucagon-like peptide 1 (GLP-1) and leptin levels.

RESEARCH DESIGN AND METHODS — A total of 10 obese nondiabetic male patients were studied before and after a 14-day treatment with 2,550 mg/day metformin and were compared with 10 untreated obese control subjects. On days 0 and 15, leptin and GLP-1(7–36)amide/(7–37) levels were assessed before and after an oral glucose load during a euglycemic hyperinsulinemic clamp to avoid the interference of variations of insulinemia and glycemia on GLP-1 and leptin secretion. The effects of metformin on GLP-1(7–36)amide degradation in human plasma and in a buffer solution containing dipeptidyl peptidase IV (DPP-IV) were also studied.

RESULTS — Leptin levels were not affected by the oral glucose load, and they were not modified after metformin treatment. Metformin induced a significant ($P < 0.05$) increase of GLP-1(7–36)amide/(7–37) at 30 and 60 min after the oral glucose load (63.8 ± 29.0 vs. 50.3 ± 15.6 pmol/l and 75.8 ± 35.4 vs. 46.9 ± 20.0 pmol/l, respectively), without affecting baseline GLP-1 levels. No variations of GLP-1 levels were observed in the control group. In pooled human plasma, metformin (0.1–0.5 μg/ml) significantly inhibited degradation of GLP-1(7–36)amide after a 30-min incubation at 37°C; similar results were obtained in a buffer solution containing DPP-IV.

CONCLUSIONS — Metformin significantly increases GLP-1 levels after an oral glucose load in obese nondiabetic subjects; this effect could be due to an inhibition of GLP-1 degradation.

Diabetes Care 24:489–494, 2001

The antihyperglycemic drug metformin has been reported to reduce body weight or prevent weight gain in obese patients with type 2 diabetes (1) and in animal models of obesity (2). The mechanism of action of metformin on body weight is still unclear; although it has been hypothesized that the drug increases energy expenditure through the enhancement of glucose turnover (3), controlled studies have consistently failed to detect a significant effect of metformin on energy expenditure (4,5). On the other hand, metformin has been reported to reduce food intake in obese rats (2) and in obese diabetic (6) and nondiabetic (7) humans. Therefore, metformin is likely to prevent weight gain primarily through an anorectic effect, the mechanism of which is unknown.

Glucagon-like peptide 1 (GLP-1), an insulinotropic hormone, has been shown to inhibit food intake and induce weight loss in humans with (8) or without (9) type 2 diabetes. The anorectic effect of GLP-1 could be attributable to both its effect on gastric emptying (9) and a direct effect on neurons in the central nervous system involved in appetite regulation (10,11). GLP-1 has been proposed as a possible therapeutic tool for type 2 diabetes and obesity (12), but its short half-life presents an obstacle to its clinical use (13). In fact, the active forms of GLP-1 (GLP-1[7–36]amide and GLP-1[7–37]) are rapidly inactivated after intravenous or subcutaneous administration (13).

Metformin has been reported to reduce leptin levels in obese nondiabetic patients (7), but this could have been the effect of weight loss, rather than a direct action of the drug on leptin secretion. Metformin has also been reported to increase glucagon-like immunoreactivity in humans (14). More recently, an increase in plasma GLP-1(7–37) levels after a test meal was observed in type 2 diabetic patients treated with preprandial metformin (15). These data suggest that metformin could increase GLP-1 circulating levels; however, the drug determines variations of blood glucose and insulin, which could interfere with GLP-1 secretion (16–18). Therefore, to avoid the interference of the effects of the drug on glucose metabolism, it is preferable to assess the action of metformin on GLP-1 secretion in isoglycemic and isoinsulinemic conditions.

The aim of this study was to assess *1*) the effects of metformin on circulating levels of leptin and GLP-1(7–36)amide/(7–37) in obese nondiabetic patients under isoglycemic and isoinsulinemic conditions and *2*) the effects of metformin

From the [1]Section of Endocrinology, Department of Clinical Pathophysiology, University of Florence; and the [2]Clinical Chemistry Laboratory, Careggi General Hospital, Florence, Italy.

Address correspondence and reprint requests to Prof. Carlo M. Rotella, Insegnamento di Malattie Metaboliche e del Ricambio, Dipartimento di Fisiopatologia Clinica, Viale Pieraccini, 6-50134 Firenze, Italy. E-mail: c.rotella@dfc.unifi.it.

Received for publication 13 July 2000 and accepted in revised form 10 November 2000.

C.M.R. is a member of the International Advisory Board on GLP-1 analogs of Novo Nordisk, Denmark. He has received honoraria from Molteni Farmaceutici, Firenze, and Bayer Farmaceutici, Milan, Italy, which are currently marketing metformin in Italy. E.M. is a paid consultant for Molteni Farmaceutici.

Abbreviations: ANOVA, analysis of variance; CV, coefficient of variation; DPP-IV, dipeptidyl peptidase IV; GLP-1, glucagon-like peptide 1.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

on GLP-1(7–36)amide degradation in human plasma.

RESEARCH DESIGN AND METHODS

— We studied 20 male obese (BMI >30 kg/m^2) patients, aged 18–45 years, with normal fasting glycemia and normal glucose tolerance who had been referred to the outpatient clinic of the Section of Metabolic Diseases, Careggi General Hospital, Florence, Italy, for the treatment of obesity. Female patients were excluded to avoid the interference of fluctuations of sex hormone levels on leptin secretion (19). Subjects with known gastrointestinal disease; hypothyroidism; or hepatic, renal, or respiratory insufficiency were not included in the study. Also excluded were those with levels of serum creatinine >1.2 mg/dl, thyrotropin >4.0 mU/l, fasting plasma glucose >6.3 mmol/l, or plasma glucose >7.7 mmol/l at 120 min after a 75-g oral glucose load. None of the patients underwent any pharmacological treatment in the 2 weeks preceding the study. All patients gave their written informed consent before their enrollment. The study protocol was approved by the Ethical Committee of the University of Florence.

Treatment

Patients were randomized to either the active treatment group or the control group. Patients in the active treatment group were treated with metformin 2,550 mg/day (850 mg three times a day after breakfast, lunch, and dinner) for 14 days. Patients in the control group did not receive any treatment throughout the duration of the study. The active treatment group and the control group were not significantly different in terms of age (46.3 ± 10.9 vs. 48.2 ± 11.2 years), BMI (34.8 ± 3.1 vs. 34.6 ± 3.2 kg/m^2), waist circumference (116.5 ± 10.9 vs. 115.8 ± 14.3 cm), and fasting and 2-h postload glycemia (5.5 ± 0.6 vs. 5.5 ± 0.7 mmol/l and 6.2 ± 1.0 vs. 6.1 ± 1.3 mmol/l, respectively). In both groups, GLP-1 and leptin levels were studied on days 0 and 15 to verify changes induced by metformin therapy.

Patients were enrolled at their first visit at the outpatient clinic, and none of the patients received a specific treatment for obesity (e.g., dietary prescription, educational programs, etc.) throughout the duration of the study.

Assessment of leptin and GLP-1 levels

Leptin and GLP-1 levels were assessed on day 0 (before the beginning of metformin therapy in the active treatment group) and day 15. Plasma leptin and GLP-1 levels were measured in the fasting state and after an oral glucose load; the assessment was performed during a euglycemic hyperinsulinemic clamp (20) to avoid metformin-induced variations in blood glucose or insulin interfering with the results.

After a 12-h overnight fast, an antecubital vein was cannulated for both the infusion of 300 mU/ml human insulin (Actrapid HM; Novo Nordisk, Copenhagen) in 0.9% NaCl and the infusion of 20% glucose. The insulin infusion rate was 127.5–45.0 mU · m^{-2} · min^{-1} for the first 10 min and then 40.0 mU · m^{-2} · min^{-1}. The glucose infusion rate was 2 mg · kg^{-1} · min^{-1} from the 4th to the 10th min and then 2.5 mg · kg^{-1} · min^{-1}. At the 10th min and every 5 min thereafter, blood glucose was determined by the glucose oxidase method (Beckman Analyser; Beckman, Brea, CA) in samples of arterialized venous blood drawn from the controlateral arm, and the glucose infusion rate was adjusted using the described algorithm (20) to achieve and maintain a glycemia of 5.5 mmol/l. The metabolic clearance rate of glucose was calculated considering the average glucose infusion rate from 75 to 90 min (20). After 90 min from the beginning of the euglycemic hyperinsulinemic clamp, an oral glucose load of 50 g in a 50% aqueous solution was administered. The glucose infusion rate was subsequently adjusted on the basis of measured glucose levels to maintain glycemia at 5.5 mmol/l. Blood samples were collected for the measurement of insulin, C-peptide, leptin, and GLP-1(7–36)amide/(7–37). Blood was drawn immediately before the beginning of the clamp, before the oral glucose load, and at 30, 60, and 90 min after the oral glucose load. For the measurement of GLP-1(7–36)amide/(7–37), blood samples were drawn into EDTA-evacuated tubes, with the addition of 30 μl (10 μl per milliliter of blood) of dipeptidyl peptidase IV (DPP-IV) inhibitor (LC0014; Linco, St. Charles, MO). The plasma was immediately separated by centrifugation at 4°C and stored at −80°C until assay.

Inhibition of GLP-1 degradation in vitro

The effect of metformin on GLP-1(7–36)amide degradation in vitro was assessed in pooled human plasma from healthy volunteers, as previously described (21). Human plasma was obtained from 11 (6 male and 5 female) healthy, lean (BMI <27 kg/m^2), nondiabetic volunteers aged 25–42 years. After an overnight fast, blood was collected at 8:30 A.M. in 10-ml EDTA-evacuated tubes with the addition of 500 UI of aprotinin A (Trasylol; Bayer Farmaceutici, Milan, Italy). The plasma was immediately separated by centrifugation at 4°C, and 1-ml samples were incubated at 37°C for 30 min with 110 pmol/l synthetic GLP-1(7–36)amide (Peninsula Laboratories, Belmont, CA) in 0.1 mol/l Tris-HCl [pH 8] and with different concentrations (0, 0.01, 0.05, 0.1, and 0.5 μg/ml) of metformin (Molteni Farmaceutici, Firenze, Italy) for 0 and 30 min (n = 6 samples for each experimental point). The reaction was halted by the addition of 20 μl DPP-IV inhibitor LC0014.

To verify whether the effects of metformin on GLP-1(7–36)amide degradation in vitro could be mediated through an inhibition of DPP-IV, 110 pmol/l of GLP-1(7–36)amide (Peninsula Laboratories) in 0.1 mol/l Tris-HCl (pH 8) was incubated with 0.06 U/ml of pig kidney DPP-IV (Sigma, St. Louis, MO) and with different concentrations (0.01, 0.05, 0.1, and 0.5 μg/ml) of metformin (Molteni Farmaceutici) for 0 and 30 min (n = 6 replicates for each experimental point). The reaction was halted by the addition of 20 μl of the DPP-IV Inhibitor LC0014, and the incubates were stored at −80°C until assay. Blood samples for the determination of leptin were drawn into evacuated gel tubes, with the addition of 500 UI of aprotinin A (Trasylol). The serum was separated by centrifugation at 4°C and frozen at −80°C until assay.

Laboratory assays

GLP-1(7–36)amide/(7–37) was measured by a GLP-1 (active) enzyme-linked immunoassay kit (Linco). This assay was based on a monoclonal antibody fixed in a coated microwell plate that binds the NH_2-terminal region of active GLP-1. The concentration of active GLP-1 is proportional to the fluorescence generated by umbelliferone, which is produced by the reaction between alkaline phosphatase

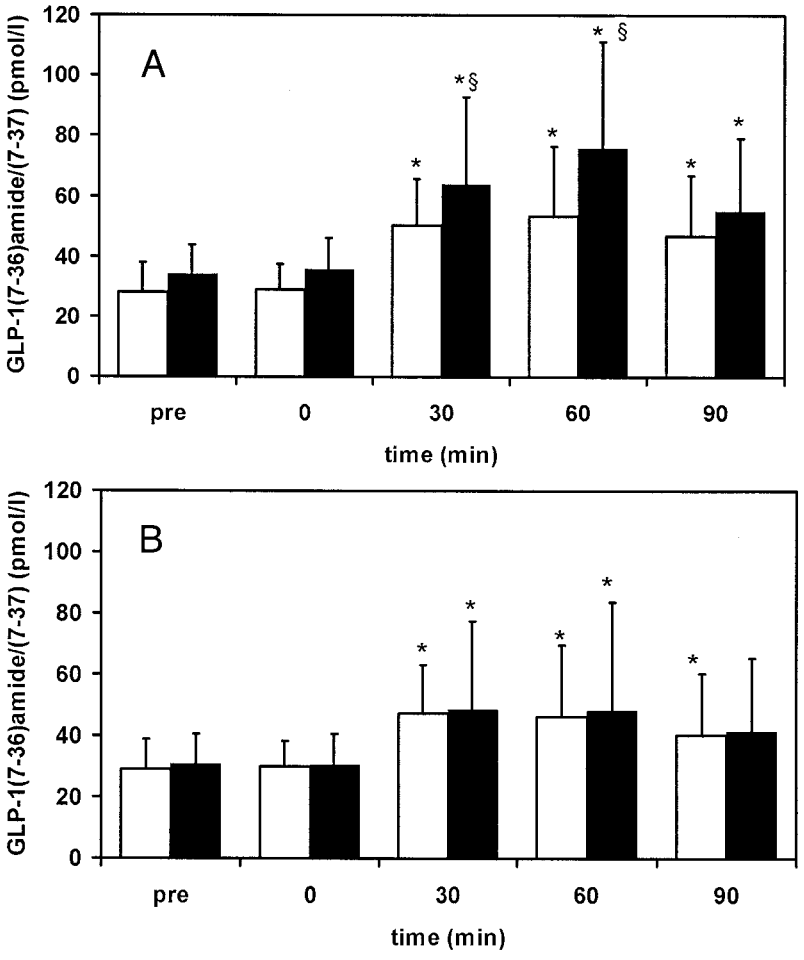

Figure 1—*Effects of metformin on GLP-1(7–36)amide/(7–37) levels. GLP-1(7–36)amide/(7–37) levels before the beginning of the clamp (pre), immediately before the oral glucose load (0), and 30, 60, and 90 min after the oral glucose load on day 0 (□) and on day 15 (■). A, active treatment group;* B, *control group. Data are expressed as means ± SD.* *P *< 0.05 vs. baseline (time 0);* §P *< 0.05 vs. before treatment (day 0).*

(conjugated with anti–GLP-1 monoclonal antibodies) and methyl umbelliferyl phosphate. The lowest reported detection limit is 2 pmol/l; the reported within-assay coefficient of variation (CV) is 8% at low and high concentrations (range 4–76 pmol/l), and the between-assay CV is 12% at 4–8 pmol/l and 7% at 28–76 pmol/l. We verified the within- and between-assay CVs, which were 12.5 and 15.5% at 8 pmol/l and 9.5 and 13.5% at 76 pmol/l, respectively. Assay cross-reactivity is 100% for GLP-1(7–36) amide and GLP-1(7–37), but it is not detectable for GLP-1(9–36)amide, GLP-2, and glucagon. Serum leptin was measured using a competitive radioimmunoassay kit for human leptin (Linco). Insulin was measured by immunoassay performed on an IMX System analyzer (Abbott Laboratories Diagnostic Division, Tokyo); C-peptide was measured by immunoassay performed on an Immulite 2000 analyzer (DPC, Los Angeles).

Statistical analysis

Data are expressed as means ± SD, except for nonnormally distributed variables, which are expressed as median (25–75th percentile). Two-tailed paired and unpaired Student's *t* tests were applied for comparison of means of variables with normal distribution, whenever appropriate; Wilcoxon's and Mann-Whitney *U* tests were applied in the case of variables with a nonnormal distribution, such as circulating insulin. One-way analysis of variance (ANOVA) was used for comparisons among more than two samples. Two-way ANOVA was used for comparisons of GLP-1 secretion among groups during stimuli. Correlations were evaluated using Pearson's method.

RESULTS

Effects of metformin on leptin and GLP-1 levels in obese patients

All patients in both the active treatment and the control groups completed the study. None of the patients experienced serious adverse events. Two patients in the active treatment group reported mild and transient diarrhea, which did not determine discontinuation of metformin therapy.

In both groups, body weight did not change significantly at the end of the study. In the metformin group, a significant improvement of insulin-mediated glucose disposal was observed after treatment (3.9 mg · kg^{-1} · min^{-1} [2.1–4.9] vs. 3.4 mg · kg^{-1} · min^{-1} [2.0–3.7], $P <$ 0.05), whereas no significant variation was observed in the control group. Insulin levels before the beginning of the clamp on day 0 and 15 in the active treatment group were 21.1 mU/l (15.6–28.6) and 17.9 mU/l (14.1–25.5), respectively (NS), whereas in the control group they were 19.2 mU/l (14.8–31.3) and 21.5 mU/l (15.9–29.8), respectively (NS vs. treatment group; NS day 15 vs. day 0). C-peptide levels before the beginning of the clamp were 1.6 ng/ml (1.2–2.1) in the active treatment group and 1.5 ng/ml (1.0–2.1) in the control group (NS) and were not significantly modified on day 15 (data not shown). In the active treatment group, on day 0 during the clamp and before the oral glucose load, insulin levels were 83.2 mU/l (75.6–97.3), and C-peptide levels were 0.7 ng/ml (0.4–0.8). Circulating insulin and C-peptide levels were not modified by the oral glucose load (data not shown). Insulin and C-peptide levels during the clamp in the control group were not significantly different from those of the active treatment group, and insulinemia and C-peptide levels during the clamp on day 15 were not significantly different from those on day 0 in both groups (data not shown).

Circulating levels of GLP-1(7–36)amide/(7–37) on days 0 and 15 are summarized in Fig. 1. Baseline and oral glucose–stimulated GLP-1(7–36)amide/(7–37) levels on day 0 were not significantly different between the two groups. Oral glucose determined a significant

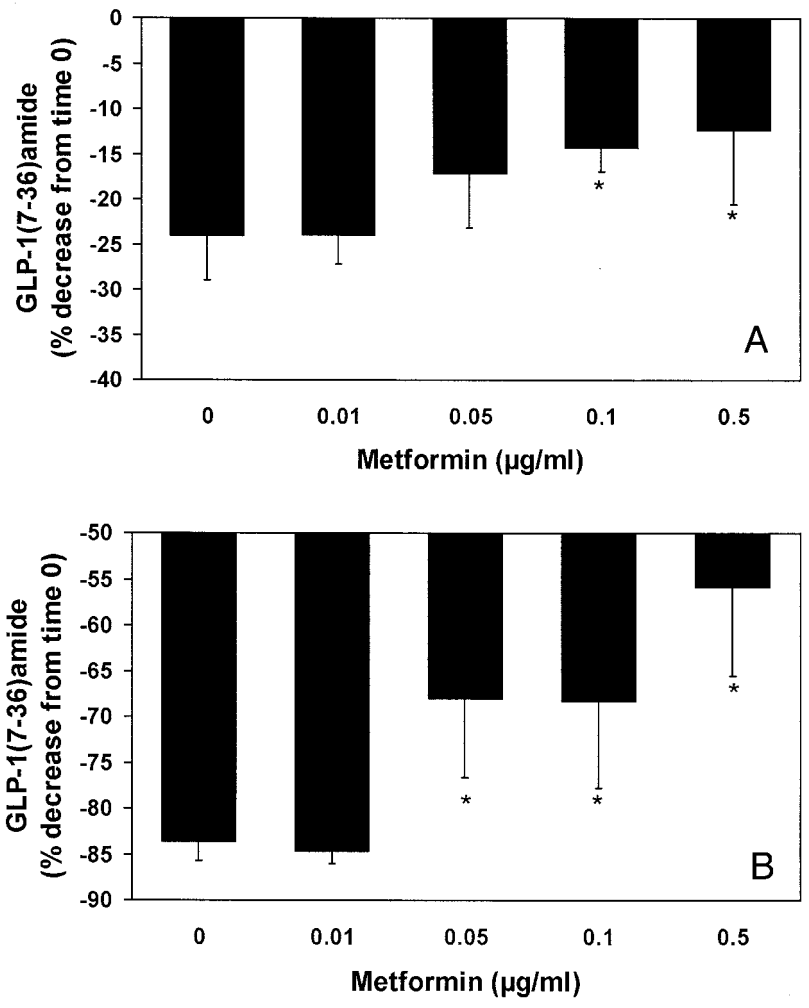

Figure 2—*Effects of metformin on GLP-1(7–36)amide degradation in vitro shown by GLP-1(7–36)amide concentrations (% variation from time 0) after a 30-min incubation with metformin in pooled sera from human volunteers* (A) *and in a buffer solution containing DPP-IV* (B)*, with added 110 pmol/l GLP-1(7–36)amide. Data are expressed as means ± SD. *P < 0.05 vs. control using Student's unpaired* t *test and ANOVA.*

($P < 0.05$, one-way ANOVA) increase of GLP-1(7–36)amide/(7–37) in both groups, with peak levels at 30 or 60 min. In both groups, peptide levels 30, 60, and 90 min after the oral glucose load were significantly higher than levels measured before the beginning of the clamp or immediately before the oral glucose load.

Metformin treatment did not induce any significant variation of GLP-1(7–36)amide/(7–37) levels before the beginning of the clamp, or immediately before the oral glucose load (Fig. 1). However, in the active treatment group, GLP-1(7–36)amide/(7–36) levels at 30 and 60 min after the oral glucose load were significantly higher on day 15 than on day 0. Peak GLP-1(7–36)amide/(7–37) levels increased significantly (93.6 ± 25.6 pmol/l after treatment vs. 63.6 ± 18.9 pmol/l before treatment, $P < 0.01$), whereas the time at which peak levels were reached was not significantly modified (48.6 ± 15.4 min after treatment vs. 42.2 ± 13.6 min before treatment, NS). No significant difference between baseline and postload peptide levels on days 15 and 0 was observed in the control group.

Two-way ANOVA confirmed a significant increase of GLP-1(7–36)amide/(7–37) after the glucose load ($P < 0.01$), a significant difference between the two groups in GLP-1(7–36)amide/(7–37) levels during the glucose challenge ($P < 0.01$), and a significant difference in the postload increase of peptide levels between the active treatment and the control groups ($P < 0.01$).

On day 0 preload leptin was 14.3 ± 6.6 ng/ml in the metformin group and 15.8 ± 6.9 ng/ml in the control group (NS). Leptin levels were significantly correlated to BMI ($r = 0.64$, $P < 0.05$). Leptin levels before the oral glucose load on day 15 were 14.8 ± 6.4 ng/ml in the metformin group and 15.2 ± 6.3 ng/ml in the control group (NS vs. day 0). Oral glucose did not induce any significant variation of leptin levels both before and after metformin therapy in the active treatment group and in the control group (data not shown).

Effects of metformin on GLP-1(7–36)amide degradation in vitro

When 110 pmol/l GLP-1(7–36)amide was added to samples of pooled sera from healthy volunteers, the measured GLP-1(7–36)amide/(7–37) concentration at time 0, in the absence of metformin, was 91.3 ± 1.4 pmol/l, with a recovery rate of 83%. The addition of 0.01–0.5 μg/ml metformin did not affect the GLP-1(7–36)amide/(7–37) concentration at time 0 (data not shown), showing that metformin does not interfere with the assay at the described doses. After a 30-min incubation at 37°C, the GLP-1(7–36)amide/(7–37) concentration was reduced by 24% when compared with time 0. Metformin in vitro inhibited GLP-1(7–36)amide degradation by human sera in a dose-dependent fashion, with significant effects at 0.5 and 0.1 μg/ml concentrations (Fig. 2*A*).

When 110 pmol/l GLP-1(7–36)amide was added to a buffer solution containing DPP IV, the measured peptide concentration at time 0 in the absence of metformin was 78.1 ± 9.2 pmol/l, with a recovery rate of 71%. The addition of 0.01–0.5 μg/ml metformin did not affect GLP-1(7–36)amide measured concentrations at time 0 (data not shown). Metformin at 0.05, 0.1, and 0.5 μg/ml significantly inhibited GLP-1(7–36)amide degradation in a dose-dependent fashion after a 30-min incubation (Fig. 2*B*).

CONCLUSIONS — Metformin treatment was generally well tolerated in obese nondiabetic patients. As expected, the drug determined a significant improvement in insulin-mediated glucose disposal. Fasting insulin levels showed a

trend toward a reduction after metformin treatment, but the differences were not statistically significant; this could be attributable to the insufficient size of the sample. No significant weight loss was observed in the present study, although a previous study had reported a significant weight loss under similar experimental conditions (7). It should be considered that the short length of treatment (2 weeks) and the small size of the sample could have prevented the observation of a significant effect on body weight. In fact, studies investigating weight loss with metformin should be designed with a larger sample size and a longer duration of treatment to obtain an adequate power to discriminate the rather small effects of the drug on body weight.

The oral glucose load did not modify leptin levels in isoglycemic or isoinsulinemic conditions, confirming previous observations (22). Metformin therapy failed to modify leptin levels; the anorectic properties of the drug do not appear to be mediated through modifications of leptin secretion.

The glucose challenge increased active GLP-1 levels during a euglycemic, hyperinsulinemic clamp. Therefore, oral carbohydrate stimulates GLP-1 secretion independently of variations in blood glucose or insulin, confirming previous observations (23).

Metformin did not modify baseline GLP-1 levels, although it significantly increased oral glucose-stimulated peptide concentrations. An increase in glucagon-like immunoreactivity had been reported in humans following metformin treatment, but the nature of that immunoreactivity was not characterized (14). More recently, metformin had been reported to increase GLP-1(7–37) levels after an oral glucose load in type 2 diabetic patients (15); however, in that uncontrolled study, the concomitant reduction in insulinemia and glycemia could have interfered with GLP-1 secretion (16–18). Moreover, that study did not assess variations in GLP-1 secretion in a control sample and did not verify the secretion of GLP-1(7–36)amide, which has much greater representation in the bloodstream than GLP-1(7–37). Present results show that metformin determines a relevant elevation of oral glucose–stimulated GLP-1(7–36)amide/(7–37), which is not dependent on variations in circulating insulin or glucose. It should be considered that the present study was not placebo-controlled; therefore, the effects of treatment on GLP-1 levels could have been at least partly attributable to the placebo effect. However, the active GLP-1 level is a parameter of which the majority of patients are not aware; therefore, it is unlikely that the placebo effect influenced the GLP-1 levels to a considerable degree. In fact, the increase of GLP-1 is not perceived by patients as an advantage, and this should limit the placebo effect.

The increase in oral glucose-stimulated GLP-1(7–36)amide/(7–37) levels determined by metformin treatment could have been due to either a stimulation of secretion or an inhibition of peptide inactivation. The circulating active forms of GLP-1 are rapidly inactivated though the cleavage of the two NH_2-terminal amino acid residues (21). The resulting peptides, GLP-1(9–37) and GLP-1(9–36)amide, lack the biological effects of the active forms of GLP-1, and they act as weak GLP-1 receptor antagonists (13). The most important enzyme involved in GLP-1 inactivation is DPP-IV, which is present in human plasma and in several tissues (13). DPP-IV inhibition has been proposed as a possible treatment for type 2 diabetes and obesity (13). The present study showed that metformin—at concentrations similar to those that can be observed in the plasma of patients treated with the usual doses of the drug (24)—inhibited GLP-1(7–36)amide degradation in human plasma and in a buffer solution containing purified DPP-IV. These results suggested that the increase in oral glucose–stimulated GLP-1 induced by metformin could have been due at least in part to a reduction of inactivation, probably through a direct inhibition of DPP-IV. However, the effects of metformin on the activity of circulating DPP-IV need to be further characterized, as well as the action of the drug on DPP-IV in tissue extracts and on other enzymes involved in GLP-1 inactivation. Moreover, the present study did not exclude the possibility that metformin also stimulates GLP-1 secretion; the effects of the drug on hormone secretion should be investigated through specifically designed studies. A further limitation of this study is the lack of current knowledge regarding possible interference with the GLP-1(1–36)amide/(1–37) assay of C-terminally extended forms of GLP-1 (e.g., major proglucagon fragment). However, these forms represent only a small fraction of circulating GLP-1 in physiological conditions; moreover, the observed inhibition of DPP-IV activity in vitro by metformin suggests that the reported increase in measured GLP-1 levels is more likely due to an increase in the active forms of the peptide.

The inhibition of GLP-1 inactivation in metformin-treated patients and the consequent increase of circulating active forms of GLP-1 after an oral glucose load could provide an explanation for the drug's weight-reducing effect, which appears to be due mainly to the inhibition of food intake (2,6,7). The increase of oral-glucose–stimulated GLP-1 concentrations could be at least partly responsible for this anorectic action. In fact, peripheral GLP-1 administration has been shown to reduce food intake in humans (8,9). The possible contribution of the increase in circulating active GLP-1 levels to other metabolic effects of metformin, such as the enhancement of insulin sensitivity (25), should also be considered and deserves further investigation.

In conclusion, treatment with metformin in obese nondiabetic patients determines a significant increase in circulating GLP-1(7–36)amide/(7–37) after an oral glucose load, but it does not affect leptin levels. The increase of GLP-1 concentrations appears to be caused at least partly by the inhibition of peptide degradation, possibly through a direct effect on DPP-IV. The effect of metformin on GLP-1 could provide an explanation for the anorectic properties of the drug. Moreover, the increase of GLP-1 levels could contribute to the antihyperglycemic effect of metformin, although this point needs further investigation.

Acknowledgments— This study was supported by grants from Molteni Farmaceutici, the Italian Ministry of University and Scientific Research (60% fund), and the Italian Ministry of Health.

References

1. Campbell IW, Howlett CS: Worldwide experience of metformin as a glucose-lowering agent: a meta-analysis. *Diabetes Metab Rev* 11 (Suppl. 1):S57–S62, 1995
2. Rouru J, Hupponen R, Pesonen U, Koulu M: Subchronic treatment with metformin produces anorectic effect and reduces hyperinsulinemia in genetically obese

Zucker rats. *Life Sci* 50:1813–1820, 1992
3. Bailey CJ: Biguanides and NIDDM. *Diabetes Care* 15:755–772, 1992
4. Stumvoll M, Nurjihan N, Perriello G, Dailey G, Gerich JE: Metabolic effects of metformin in non-insulin-dependent diabetes mellitus. *N Engl J Med* 333:550–554, 1995
5. Makkimattila S, Nikkila K, Yki-Jarvinen H: Causes of weight gain during insulin therapy with and without metformin in patients with type II diabetes mellitus. *Diabetologia* 42:406–412, 1999
6. Lee A, Morley J: Metformin decreases food consumption and induces weight loss in subjects with obesity with type II non-insulin-dependent diabetes. *Obes Res* 6:47–53, 1998
7. Paolisso G, Amato L, Eccellente R, Gambardella A, Tagliamonte MR, Varricchio G, Carella C, Giugliano D, D'Onofrio F: Effect of metformin on food intake in obese subjects. *Eur J Clin Invest* 28:441–446, 1998
8. Gutzwiller JP, Goke B, Drewe J, Hildebrand P, Ketterer S, Handshin D, Winterhalder R, Conen D, Beglinger C: Glucagon-like peptide-1: a potent regulator of food intake in humans. *Gut* 44:81–86, 1999
9. Naslund E, Gutniak M, Skogar S, Rossner S, Hellstrom PM: Glucagon-like peptide 1 increases the period of postprandial satiety and slows gastric emptying in obese men. *Am J Clin Nutr* 68:525–530, 1998
10. Ørskov C, Poulsen SS, Møller M, Holst JJ: Glucagon-like peptide I receptors in the subfornical organ and the area postrema are accessible to circulating glucagon-like peptide 1. *Diabetes* 45:832–835, 1996
11. Tang-Christensen M, Vrang N, Larsen PJ: Glucagon-like peptide-1(7–36) amide's central inhibition of feeding and peripheral inhibition of drinking are abolished by neonatal monosodium glutamate treatment. *Diabetes* 47:530–537, 1998
12. Byrne MM, Goke B: Human studies with glucagon-like peptide-1: potential of the gut hormone for clinical use (Review). *Diabet Med* 13:854–860, 1996
13. Holst JJ, Deacon CF: Inhibition of the activity of dipeptidyl-peptidase IV as a treatment for type 2 diabetes. *Diabetes* 47: 1663–1670, 1998
14. Molloy AM, Ardill J, Tomkin GH: The effect of metformin treatment on gastric acid secretion and gastrointestinal hormone levels in normal subjects. *Diabetologia* 19:93–96, 1980
15. Lugari R, Dell'Anna C, Sarti L, Gnudi A: Effects of metformin on intestinal and pancreatic endocrine secretion in type 2 (non-insulin-dependent) diabetes. In *Molecular and Cell Biology of Type 2 Diabetes and its Complications*. Belfiore F, Lorenzi M, Molinatti GM, Porta M, Eds. Basel, Karger, 1998, p. 161–163
16. Takahashi H, Manaka H, Suda K, Fukase N, Sekikawa A, Eguchi H, Tominaga M, Sasaki H: Hyperglycaemia but not hyperinsulinaemia prevents the secretion of glucagon-like peptide-1 (7–36 amide). *Scand J Lab Clin Invest* 51:499–507, 1991
17. Hermann C, Goke R, Richter C, Fehmann HC, Arnold R, Goke B: Glucagon-like peptide-1 and glucose-dependent insulin-releasing polypeptide plasma levels in response to nutrients. *Digestion* 56:117–126, 1995
18. Ørskov C, Rabenhøj L, Wettergren A, Kofod H, Holst JJ: Tissue and plasma concentrations of amidated and glycine-extended glucagon-like peptide I in humans. *Diabetes* 43:535–539, 1994
19. Mannucci E, Ognibene A, Becorpi A, Cremasco F, Pellegrini S, Ottanelli S, Rizzello SM, Massi G, Messeri G, Rotella CM: Relationship between leptin and oestrogens in healthy women. *Eur J Endocrinol* 139: 198–201, 1998
20. DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 237:E214–E223, 1979
21. Deacon CF, Nauck MA, Toft-Nielsen M, Pridal L, Willms B, Holst JJ: Both subcutaneously and intravenously administered glucagon-like peptide I are rapidly degraded from the NH_2-terminus in type II diabetic patients and in healthy subjects. *Diabetes* 44:1126–1131, 1995
22. Kautzky Willer A, Ludwig C, Nowotny P, Roden A, Huerner C, Widhalm K, Vierhapper H, Waldhausl W, Roden M: Elevation of plasma leptin concentrations in obese hyperinsulinemic hypothyroidism before and after treatment. *Eur J Clin Invest* 29:395–403, 1999
23. Lavin JH, Wittert GA, Andrews J, Andrews I, Yeap B, Wishart JM, Morris HA, Morley JE, Horowitz M, Read NW: Interaction of insulin, glucagon-like peptide-1, gastric inhibitory polypeptide, and appetite in response to intraduodenal carbohydrate. *Am J Clin Nutr* 68:591–598, 1998
24. Weinsperger NF: Preclinical pharmacology of biguanides. In *Oral Antidiabetics*. Kuhlmann J, Puls W, Eds. Berlin, Springer, 1996, p. 305–358
25. D'Alessio DA, Kahn SE, Leusner CR, Ensinck JW: Glucagon-like peptide-1 enhances glucose tolerance both by stimulation of insulin release and by increasing insulin-independent glucose disposal. *J Clin Invest* 95:2263–2266, 1994