Effect of metformin on insulin sensitivity and insulin secretion in female obese patients with normal glucose tolerance

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S U M M A R Y

Objectives: Metformin is recognized as the treatment of chronic obese, insulin-resistant type 2 diabetic patients. Whether it improves insulin sensitivity in obese patients with normal glucose tolerance remains unknown.

Methods: Eight obese female patients with normal glucose tolerance were studied during a double blinded, randomized cross-over study including a 2-week administration of metformin and a 2-week administration of placebo. Insulin secretion and insulin sensitivity were assessed after metformin and placebo by means of a 3-hour hyperglycemic clamp.

Results: The plasma insulin and C-peptide concentrations during the hyperglycemic clamp were identical after placebo or metformin (both first and second phases). Insulin-mediated glucose disposal, stimulation of glucose oxidation and suppression of endogenous glucose production were identical after metformin and placebo.

Conclusions: Metformin does not improve insulin sensitivity nor insulin secretion in obese female patients with normal glucose tolerance.

Key-words: Obesity - Glucose tolerance - Insulin resistance - Glucose production.


R É S U M É

Effet de la metformine sur la sensibilité à l’insuline et l’insulinosécrétion chez la femme obèse avec tolérance au glucose normale

Objectif : La metformine constitue le traitement de premier choix du patient obèse diabétique de type 2. Son effet sur la résistance à l’insuline chez le patient obèse avec tolérance au glucose normale n’est cependant pas documenté.

Méthodes : Huit patientes obèses avec tolérance au glucose normale ont été étudiées en double aveugle dans une étude en cross-over incluant 2 semaines de traitement par la metformine ou en placebo. La sécrétion d’insuline et la sensibilité à l’insuline de ces patients ont été mesurées par la méthode du clamp hyperglycémique.

Résultats : Pendant le clamp hyperglycémique, les concentrations d’insuline et de peptide C étaient semblables après metformine et placebo. L’utilisation totale du glucose, l’oxydation nette des hydrates de carbone et l’inhibition de la production endogène de glucose étaient aussi identiques après metformine ou placebo.

Conclusions : La metformine ne modifie pas la sensibilité à l’insuline, ni l’insulino-sécrétion chez des patientes obèses avec une tolérance au glucose normale.

Mots-clés : Obésité - Tolérance au glucose - Résistance à l’insuline - Production de glucose.
Introduction

Metformin is widely used for the treatment of type 2 diabetes mellitus. Although its mechanism of action remains debated, it is thought to reduce hyperglycemia essentially by suppressing hepatic gluconeogenesis and decreasing endogenous glucose production [1, 2]. It has also been suggested that an improved insulin sensitivity may contribute to this effect.

Insulin resistance is also encountered in many non diabetic individuals and is associated with obesity, dyslipidemia, high blood pressure and is recognized as an independent cardiovascular risk factor [3]. Treatment of these conditions with metformin has been advocated, and a large clinical trend is presently underway to evaluate the effects of this drug on metabolic and cardiovascular parameters [4, 5].

The effects of metformin on glucose metabolism in non diabetic, insulin-resistant individuals have, however, been little investigated. We therefore assessed, in a group of obese female patients with normal glucose tolerance, the effects of a two-week treatment with metformin on glucose induced insulin secretion, insulin sensitivity, and glucose metabolism. Since biguanids have been associated with the rare occurrence of lactic acidosis [6], we also evaluated its effect on plasma lactate levels and on adipose and skeletal muscle lactate production.

Methods

Subjects

Eight obese female patients (mean age 37.8 ± (sem) 2.1 years, mean weight 86.8 ± 45 kg, mean height 1.63 ± 0.02 m, mean BMI 32.5 ± 1.3 kg/m²) were recruited to take part in this study. All were in apparent good health except obesity; they were not taking any medication and had maintained a constant body weight for at least 3 months. They all provided an informed, written consent. They all had a normal glucose tolerance, as documented by a standard, 75 g oral glucose tolerance test.

Experimental protocol

Each subject was submitted to a two-week treatment of metformin 3 x 850 mg/day or placebo 3 x 850 mg/day, followed by a 6-week washout period and a second two-week treatment with either placebo or metformin (crossover, randomized design). Empty tablet containers were collected to evaluate compliance. The whole study was done in a double-blinded fashion.

At the end of each two-week period of treatment with metformin or placebo, every subject underwent a 5-hour metabolic investigation which was performed during the follicular phase of a menstrual cycle. For each investigation, the subjects reported to the metabolic investigation laboratory of Lausanne University School of Medicine at 7 AM in a fasting state. At their arrival, they took their last tablet of either metformin or placebo; they were then requested to void, and took then place in a bed in a semirecumbent position. A venous cannula was inserted into a vein of their right wrist for collection of blood samples. Their right hand was placed in a thermostabilized box heated at 50°C in order to achieve partial arterialization of the collected venous blood. Another venous cannula was inserted into a forearm vein on the left side. Through this cannula, a primed (2 mg/kg), continuous (20 µg/kg/min) infusion of 6.6 2H2 glucose (Masstrace, Worcester, MA) was started at time -120 min and continued until time 180 min. At time 0, a 3-hour hyperglycemic clamp was started [7]. A variable infusion of 20% dextrose was administered to acutely raise and maintain glycemia at 180 mg/dl between 0 and 180 min. The infused dextrose was labeled with 1.25% 6.6 2H2 glucose. Blood samples were obtained at time -30, 0, 2, 4, 6, 8, 10, 15, 30, 60, 90, 120, 150 and 180 min for determination of hormone substrates and tracer concentrations.

Two microdialysis catheters (CMA, Stockholm, Sweden) were inserted, one into the subcutaneous adipose tissue of the abdomen and the other in the muscle tibialis anterior. These catheters were continuously infused with a Ringer solution containing 1 mmol/l 3H3 lactate (Cambridge Isotopes, Cambridge, MA). The effluent dialysate was collected as 30 min fractions from – 30 to 180 min. From -60 min to 180 min, O2 and CO2 respiratory exchanges were continuously monitored by indirect calorimetry, using a ventilated hood system, as previously described [8]. A urine collection was obtained at the end of the experiment to measure urinary urea excretion rate.

Analytical procedures

Plasma glucose concentrations were obtained at the bedside using a Beckman glucose analyzer II (Beckman Instruments, Brea, CA). Plasma lactate concentrations were measured enzymatically, using a YSI lactate analyzer (Yellow Spring Instruments, Yellow Springs, OH). Plasma free fatty acids were measured colorimetrically, using a kit from WAKO, Freiburg, Germany. Plasma insulin and C-peptide concentrations were measured by radioimmunoassay, using kits from LINCO (St Charles, MO). Concentrations of glucose and lactate in the dialysate were measured enzymatically, using a CMA microdialysis analyzer. Plasma 6,6 2H2 glucose and dialysate 3H3 lactate were measured by gas chromatography mass spectrometry, as described previously [9, 10].

Calculations

Whole body glucose turnover was calculated from plasma 6,6 2H2 glucose dilution analysis, using “hot infusate” equations [11].

Endogenous glucose production was calculated as (whole body glucose turnover) - (exogenous infusion).
Whole body insulin sensitivity was calculated as (whole body glucose turnover) . (plasma insulin concentration)⁻¹.

Whole body glucose turnover and plasma insulin concentration values were averaged over the period 150-180 min for this calculation.

The recovery of lactate through the dialysis membrane was calculated as

\[ \frac{[\text{\textsuperscript{2}H\textsubscript{5} lactate]_{\text{out}}}}{[\text{\textsuperscript{2}H\textsubscript{5} lactate]_{\text{in}}}} \]

The interstitial lactate concentration in adipose tissue and skeletal muscle were calculated by dividing the concentrations in the dialysate by the corresponding recovery factor.

Net carbohydrates and lipid oxidation rates were calculated from respiratory gas exchanges and urinary urea excretion rate, using the equations of Livesey and Elia [12].

Figure 1
Plasma glucose, insulin, and C-peptide concentrations in obese patients with normal glucose tolerance during a 3-hour hyperglycemic clamp. Open circles indicate placebo administration; closed circles indicate metformin treatment.
Statistical analysis

The effects of metformin on the various parameters measured were assessed by a two-way analysis of variance and paired t-tests with Bonferroni’s adjustment.

Results

All patients had fasting plasma glucose concentrations in the normal range after both placebo and metformin (Fig 1). Their fasting insulin (19.5 ± 2.9 mU/l) and C-peptide concentrations (3.3 ± 0.5 µg/l) were mildly elevated compared to values observed in healthy lean volunteers (insulin 9.1 ± 1.0 mU/l, C-peptide 1.6 ± 0.2 µg/l) in our institution but were identical after placebo or metformin (Fig 1).

During the hyperglycemic clamp, plasma glucose concentrations were maintained close to the target value of 180 mg/dl from 30 to 180 min after both placebo and metformin. First phase insulin (0-10 min) secretion was identical after placebo and metformin. The plasma concentrations attained during the second phase of insulin secretion were identical after metformin and placebo until 120 min. Thereafter, they were slightly lower after metformin. This was due essentially to very high insulin concentrations attained during this period in one patient after placebo and the difference was not statistically significant. A similar trend was observed with C-peptide concentrations (Fig 1). Plasma free fatty acid concentrations were 0.599 ± 0.068 vs 0.606 ± 0.041 mmol/l in basal conditions and 0.148 ± 0.019 vs 0.197 ± 0.021 mmol/l, placebo vs metformin at the end of the clamp (all ns).

The exogenous glucose infusion rates required to maintain glycemia at a steady value of 180 mg/dl were absolutely identical after placebo or metformin (Fig 2). The whole body glucose rates of disappearance were also similar after placebo or metformin (Fig 2). Endogenous glucose production measured in basal condition was comparable after metformin and placebo and was suppressed to the same extent during the last hour of the hyperglycemic clamp (Fig 3).

C Binnert et al.
Diabetes Metab 2003, 29, 125-32 • www.e2med.com/dm
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Insulin sensitivity was assessed by calculating the ratio of total glucose disappearance rate: plasma insulin concentrations during the last 60 min of the clamp (time 120-180 min). There was no difference in insulin sensitivity after metformin compared to placebo (Fig 4). There was also no effect of metformin on net carbohydrate and lipid oxidation rates, nor on total energy expenditure (Fig 5).

After placebo administration, plasma lactate increased significantly by 50% after 1 hour and declined thereafter. Until the end of the clamp, it remained higher than baseline.
values (Fig 6). The interstitial concentrations of lactate also increased in adipose tissue and skeletal muscle during the clamp. After metformin, the plasma lactate concentrations were on average 9% higher throughout the experiment compared to placebo. The difference was, however, not significant. The same trend was observed for interstitial lactate (Fig 6).

**Discussion**

This study was designed to evaluate the effects of metformin on insulin secretion and insulin sensitivity in a group of obese female patients with normal glucose tolerance. The major observation is that metformin did not increase insulin secretion nor enhanced insulin sensitivity in this subgroup of obese patients. In particular, metformin did not change first phase insulin secretion which is the earliest observed alteration in patients with impaired glucose tolerance [13]. The study participants were all insulin-resistant, as indicated by increased fasting plasma insulin concentrations and by decreased glucose rate of disappearance: insulin ratios compared to values obtained in healthy lean individuals in our laboratory [14, 15]. They had, however, a normal glucose tolerance, as documented by a standard oral glucose tolerance test.

Our result appears at odds with several reports indicating that metformin improves glucose tolerance and enhances insulin sensitivity in obese and non-obese insulin-resistant patients with impaired glucose tolerance [16-19]. This may be explained by the fact that the patients included in these studies were morbidly obese or were more severely insulin-resistant. Alternatively, there is evidence that the development of impaired glucose tolerance is tightly associated with impaired post-prandial suppression of glucose production in both obese and non-obese individuals [20]. An improvement by metformin of insulin-mediated glucose metabolism in patients with impaired glucose tolerance is therefore consistent with an effect exerted primarily at the level of the liver [21] to suppress endogenous glucose production. Our results also appear at odds with reports showing an improved insu-
In sensitivity after metformin treatment in patients with polycystic ovaries syndrome [22-25]. It is likely, however, that this subgroup of patients was more severely insulin-resistant. In addition, metformin may possibly have effects on sex steroid secretion and actions.

Given the rare occurrence of hyperlactatemia and lactic acidosis associated with biguanids, we also monitored plasma and adipose/skeletal muscle interstitial lactate concentrations. Metformin treatment had no significant effects on plasma lactate concentrations. Interstitial lactate concentrations were always higher in adipose tissue and skeletal muscle, indicating that these two tissues contribute significantly to systemic lactate production [26-28]. In both tissues, interstitial lactate concentrations tended to increase after
metformin treatment, but the difference was far from reaching statistical significance. These results indicate that metformin has little effect on lactate production and plasma lactatemia in obese, insulin-resistant patients without alterations of hepatic glucose metabolism. They suggest that metformin may increase lactate concentrations by decreasing hepatic lactate utilization (i.e., gluconeogenesis) in patients with impaired glucose tolerance, but does not stimulate per se non oxidative glycolysis in extrahepatic tissues.

Altogether, our results indicate that metformin does not increase glucose-induced insulin secretion and does not induce detectable effects on insulin sensitivity in obese patients with normal glucose tolerance. It may therefore have little beneficial effects in the absence of the alterations of hepatic glucose metabolism which characterize patients with impaired glucose tolerance.

Acknowledgements – This work was supported by a grant from Merck Lipha, Lyon, France.

References