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Direct comparison among oral hypoglycemic agents and their association with insulin resistance evaluated by euglycemic hyperinsulinemic clamp: the 60's study

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Abstract

The aim of the study was to compare the long-term effect of 4 antidiabetic treatment protocols on insulin resistance evaluated by euglycemic hyperinsulinemic clamp in type 2 diabetes mellitus patients. Two hundred seventy-one type 2 diabetes mellitus patients with poor glycemic control and who were overweight were enrolled in this study. Patients were randomized and titrated to take pioglitazone, metformin, pioglitazone + metformin, or glimepiride + metformin for 15 months. They underwent a euglycemic hyperinsulinemic clamp at baseline, after 3 months, and after 15 months. Anthropometric and metabolic measurements were assessed at baseline, after 3 months, and after 15 months. There was a decrease in glycated hemoglobin in all groups, but glycated hemoglobin value was lower in the group treated with pioglitazone + metformin compared with the groups treated with metformin alone and with pioglitazone alone. There was a decrease in fasting plasma glucose and postprandial plasma glucose values in all groups, but values obtained with pioglitazone + metformin were lower compared with values in the groups treated with metformin alone and with pioglitazone alone. Fasting plasma insulin and postprandial plasma insulin values were higher in the group treated with glimepiride + metformin compared with the other groups. After 15 months, glucose infusion rate and total glucose requirement values observed in the groups treated with pioglitazone alone and with pioglitazone + metformin were higher compared with the values in the group treated with metformin alone and with glimepiride + metformin; furthermore, values obtained in the group treated with pioglitazone + metformin were higher than the value obtained with pioglitazone alone. Pioglitazone-metformin–based therapeutic control is associated with the most quantitatively relevant improvement in insulin resistance–related parameters, whereas the sulfonylurea-metformin–including protocol has less relevant effects.

1. Introduction

Insulin resistance is currently accepted to be a major risk factor in the etiology of type 2 diabetes mellitus, even in individuals with normal glucose tolerance [1], and in most strong independent cardiovascular disease risk factors such as hypertension [2], dyslipidemia [3], and atherosclerosis [4]. Moreover, insulin resistance is also associated with the development of the nonalcoholic fatty liver disease that is currently under investigation as a new cardiovascular disease risk factor [5]. Therefore, in some studies, insulin resistance appears to be per se an independent risk factor for coronary artery disease [6] and stroke [7].

On the other side, the only antidiabetic agents associated with a reduction of cardiovascular disease morbidity and mortality are those that directly or indirectly improve insulin resistance, such as metformin [8], pioglitazone [9], and acarbose [10], respectively.

The study protocol was approved at each site by institutional review boards and was conducted in accordance with the Declaration of Helsinki. All patients provided written informed consent.

The authors certify that they have no affiliation with or financial involvement in any organization or entity with a direct financial interest in the subject matter or materials discussed in the manuscript.

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Glimepiride, the latest second-generation sulfonylurea for treatment of type 2 diabetes mellitus, is a safe direct insulin secretagogue; but it also indirectly increases insulin secretion in response to fuels such as glucose [11].

Even if during the last 20 years various indices of insulin sensitivity/resistance mainly based on plasma glucose and insulin level have been proposed, the euglycemic hyperinsulinemic clamp remains the criterion standard to evaluate this parameter [12].

The aim of this study was to directly compare the long-term effect of 4 antidiabetic treatment protocols on insulin resistance evaluated by euglycemic hyperinsulinemic clamp in type 2 diabetes mellitus patients. In particular, we aimed to evaluate if the combination of 2 insulin-sensitizing agents (pioglitazone and metformin) could significantly improve the insulin resistance when compared with single agent–based protocols and with a protocol including an insulin secretagogue (glimepiride).

2. Materials and methods

2.1. Study design

This multicenter, double-blind, randomized, controlled trial was conducted in the Department of Internal Medicine and Therapeutics at the University of Pavia and in the "G Descovich" Atherosclerosis Study Center, "D Campanacci" Clinical Medicine and Applied Biotechnology Department, University of Bologna. At baseline, naive type 2 diabetes mellitus patients underwent a euglycemic hyperinsulinemic clamp; and after a 3-month period in which these patients were randomized and titrated (forced titration, independently from their glycemic control, unless they developed adverse effects also due to the drug dosage) to take pioglitazone, metformin, pioglitazone + metformin, or glimepiride + metformin, they underwent a second euglycemic hyperinsulinemic clamp. The patients were then followed for 12 months. At the end of this period, patients underwent a third euglycemic hyperinsulinemic clamp (Fig. 1). Anthropometric and metabolic measurements were assessed at baseline, after 3 months, and after 12 months.

2.2. Patients

We enrolled 271 white patients, at least 18 years of age of either sex (Table 1), with type 2 diabetes mellitus according to the European Society of Cardiology and the European Association for the Study of Diabetes guidelines criteria [13] who were naive and with poor glycemic control, expressed as glycated hemoglobin (HbA_{1c}) level greater than 6.5%, and were overweight (body mass index [BMI] $\geq$25 and <30 kg/m^2). Suitable patients, identified from review of case notes and/or computerized clinic registers, were contacted by the investigators in person or by telephone.

Patients were excluded if they had a history of ketoacidosis or had unstable or rapidly progressive diabetic retinopathy, nephropathy, or neuropathy; *impaired hepatic function* (defined as plasma aminotransferase and/or γ-glutamyltransferase level higher than the upper limit of normal for age and sex); *impaired renal function* (defined as serum creatinine level higher than the upper limit of normal for age and sex); or severe anemia. Patients with serious cardiovascular disease (eg, New York Heart Association class I-IV congestive heart failure or a history of myocardial infarction or stroke) or cerebrovascular conditions within 6 months before study enrollment also were excluded. Women who were pregnant, breastfeeding, or of childbearing potential and not taking adequate contraceptive precautions were also excluded. All patients provided written informed consent to participate.

2.3. Treatments

Patients were randomly assigned to receive pioglitazone (15 mg/d; once a day, after lunch), metformin (1000 mg/d; 500 mg, twice a day, after lunch and dinner), pioglitazone + metformin (15 + 850 mg/d; once a day, after lunch), or glimepiride + metformin (2 + 850 mg/d; once a day, after lunch). Randomization was done using a drawing of envelopes containing randomization codes prepared by a statistician. A

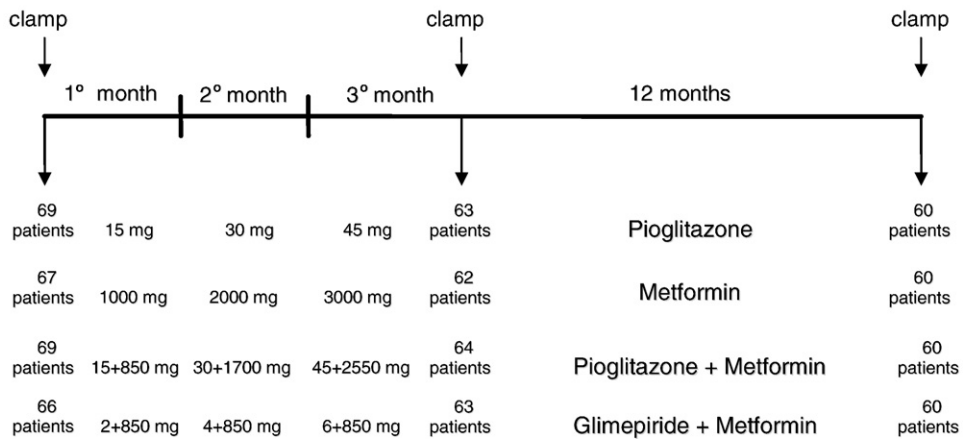

Fig. 1. Study design.

Table 1
Type 2 diabetes mellitus patients' characteristics at baseline in the study

	Pioglitazone	Metformin	Pioglitazone + metformin	Glimepiride + metformin
n	69	67	69	66
Sex (M/F)	32/37	34/33	34/35	32/34
Age (y)	54 ± 6	55 ± 5	57 ± 7	57.7 ± 7
Smoking status (n)	16	18	19	15
Height (m)	1.67 ± 0.03	1.69 ± 0.05	1.67 ± 0.04	1.69 ± 0.05
Weight (kg)	76.7 ± 5.3	77.7 ± 5.9	76.4 ± 5.1	77.4 ± 5.8
BMI (kg/m^2)	27.5 ± 1.7	27.2 ± 1.5	27.4 ± 1.6	27.1 ± 1.4
HbA_{1c} (%)	9.2 ± 1.3	9.1 ± 1.2	9.3 ± 1.4	9.0 ± 1.1
FPG (mg/dL)	164 ± 28	161 ± 27	167 ± 29	168 ± 30
PPG (mg/dL)	197 ± 41	192 ± 38	198 ± 42	199 ± 44
FPI (μU/mL)	24.8 ± 6.4	24.6 ± 6.3	25.2 ± 6.6	23.9 ± 6.0
PPI (μU/mL)	79.5 ± 21.7	81.6 ± 22.2	84.6 ± 23.5	84.9 ± 23.9
GIR (μmol/[min kg])	5.83 ± 0.68	5.71 ± 0.63	5.73 ± 0.64	5.64 ± 0.56
TGR (μmol/[min kg])	34.8 ± 6.4	34.1 ± 6.2	34.6 ± 6.3	33.2 ± 5.8

Data are means ± SD unless otherwise specified. *P* = nonsignificant.

copy of the code was provided only to the responsible person performing the statistical analysis. The code was only broken after database lock, but could have been broken for individual subjects in cases of an emergency. Medication compliance was assessed by counting the number of pills returned at the time of specified clinic visits. The treatments were supplied as matching opaque white capsules in coded bottles to ensure the double-blind status of the study. At baseline, we weighed participants and gave them a bottle containing a supply of study medication for at least 100 days. Throughout the study, we instructed patients to take their first dose of new medication on the day after they were given the study medication. A bottle containing the new study medication for the next treatment period was given to participants every 3 months. At the same time, all unused medications were retrieved for inventory. All medications were provided free of charge. After this enrolment, every month for 3 months, the treatment in each arm was titrated, as shown in Fig. 1; and the patients were followed for 12 months.

The study protocol was approved at each site by institutional review boards and was conducted in accordance with the Declaration of Helsinki. All patients provided written informed consent.

2.4. Diet and exercise

At baseline, patients began a controlled-energy diet (~600 kcal daily deficit) based on American Diabetes Association recommendations [14] that contained 50% of calories from carbohydrates, 30% from fat (6% saturated), and 20% from proteins, with a maximum cholesterol content of 300 mg/d, and 35 g/d of fiber. Each center's standard diet advice was given by a dietitian and/or specialist physician. Dietitians and/or specialists each month for the first 3 months provided instruction on dietary intake, recording procedures as part of a behavior-modification program, and then from month 3 used the patients' food diaries for counseling. During the study, behavior-modification sessions on weight-loss strategies were given to individual patients at baseline and then every 3 months until the end of the trial. Individuals were also encouraged to increase their physical activity by walking briskly or riding a stationary bicycle for 20 to 30 minutes, 3 to 5 times per week. The recommended changes in physical activity throughout the study were not assessed.

2.5. Assessments

Before starting the study, all patients underwent an initial screening assessment that included a medical history; physical examination; vital signs; a 12-lead electrocardiogram; and measurements of BMI, HbA_{1c}, fasting plasma glucose (FPG), postprandial plasma glucose (PPG), fasting plasma insulin (FPI), and postprandial plasma insulin (PPI).

All plasmatic parameters were determined after a 12-hour overnight fast, except that PPG and PPI were determined 2 hours after lunch. Venous blood samples were taken for all patients between 8:00 and 9:00 AM. We used plasma obtained by addition of Na_2-EDTA, 1 mg/mL, and centrifugation at 3000*g* for 15 minutes at 4°C. Immediately after centrifugation, the plasma samples were frozen and stored at −80°C for no more than 3 months. All measurements were performed in a central laboratory.

Body mass index was calculated as weight in kilograms divided by the square of height in meters. Glycated hemoglobin level was measured by a high-performance liquid chromatography method (DIAMAT; Bio-Rad, Hercules, CA; normal values, 4.2%-6.2%), with intra- and interassay coefficients of variation of less than 2% [15]. Plasma glucose was assayed by glucose-oxidase method (GOD/PAP; Roche Diagnostics, Mannheim, Germany) with intra- and interassay coefficients of variation of less than 2% [16]. Plasma insulin was assayed with Phadiaseph Insulin Radioimmunoassay (Pharmacia, Uppsala, Sweden) by using a second antibody to separate the free and antibody-bound 125 I-insulin (intra- and interassay coefficients of variation, 4.6% and 7.3%, respectively) [17].

Body mass index, HbA_{1c}, FPG, PPG, FPI, and PPI were evaluated at baseline, after 3 months, and after 15 months.

To evaluate the tolerability assessments, all adverse events were recorded.

2.6. Insulin sensitivity evaluation

Insulin sensitivity was assessed with the use of the euglycemic hyperinsulinemic clamp, according to the technique of De Fronzo et al [18]. At 9:00 AM, after the subjects had fasted for 12 hours overnight, an intravenous catheter (18-gauge polyethylene cannula; Venflon, Viggo, Helsingborg, Sweden) was placed in an antecubital vein for infusion of insulin and 20% glucose. A second catheter was inserted retrogradely into a wrist vein. The hand was heated (about 70°C) in a thermoregulated box with the aim of arterializing venous blood within 20 to 40 minutes [19]. Plasma glucose was assessed at 5- to 10-minute intervals during the clamp. A 10-minute priming infusion of insulin (Humulin R; Eli Lilly, Indianapolis, IN) was administered at rate of 1 mU/(min kg) for 2 hours, during which the plasma glucose concentration was held constant at the basal state (95 mg/dL) by a variable infusion of exogenous glucose. The amount of glucose required to maintain isoglycemia equals whole-body disposal of glucose, provided that endogenous glucose production is essentially absent. During insulin infusion, normal fasting blood glucose levels were maintained by adjustment of the infusion of a 20% glucose solution. The amount of glucose taken up (micromoles per minute per kilogram of body weight) was calculated for each 10-minute interval after the first 20 minutes of the clamp. Insulin sensitivity was calculated from the mean glucose uptake rate for the last 30 minutes of the clamp and expressed as the amount of glucose infused during that time (glucose infusion rate [GIR]) in micromoles per minute per kilogram of body weight.

The total amount of exogenous glucose required to maintain a steady-state blood glucose level in response to a defined increase in plasma insulin concentration (whole-body glucose disposal rates [total glucose requirement, TGR]) was also evaluated.

2.7. Statistical analysis

An intent-to-treat analysis was conducted in patients who had received at least 1 dose of study medication and had a subsequent efficacy observation. Patients were included in the safety analysis if they had received 1 dose of trial medication after randomization and had a subsequent safety observation. The null hypothesis that the expected mean BMI, HbA_{1c}, FPG, PPG, FPI, PPI, GIR, and TGR change from baseline to the end of 15 months of double-blind treatment did not differ significantly among the treatments was tested using repeated-measures analysis of variance and analysis of covariance models [20]. The statistical significance of the independent effects of treatments on the other parameters was determined by analysis of covariance. A 1-sample *t* test was used to compare values obtained before and after treatment administration, and 2-sample *t* tests were used for between-group comparison. The Bonferroni correction for multiple comparison also was carried out. Differences over time and association between BMI and HbA_{1c} levels with GIR or other variables were evaluated with stepwise multiple linear regression analysis.

Considering as clinically significant a difference of at least 10% compared with the baseline and an α error of .05, the actual sample size is adequate to obtain a power higher than 0.80 for all variables related to glucose metabolism (HbA_{1c}, FPG, PPG, FPI, PPI, GIR, and TGR).

Statistical analysis of data was performed by means of the SPSS (Chicago, IL) statistical software package for Windows (version 11.0); data are presented as mean ± SD. For all statistical analyses, *P* less than .05 was considered statistically significant.

3. Results

3.1. Study sample

A total of 271 (132 men and 139 women) patients (69 patients in pioglitazone group, 67 patients in metformin group, 69 patients in pioglitazone + metformin group, and 66 patients in glimepiride + metformin) were enrolled in the study. Two hundred fifty-two (125 men and 127 women) patients (63 patients in pioglitazone group, 62 patients in metformin group, 64 patients in pioglitazone + metformin group, and 63 patients in glimepiride + metformin) completed the titration phase. The reasons for premature withdrawal included being lost to follow-up (1 man and 1 woman in pioglitazone group, 2 women in metformin group, 1 man and 1 woman in pioglitazone + metformin, and 1 man in glimepiride + metformin), protocol violation (1 woman in pioglitazone group, 1 woman in metformin group, 1 man and 1 woman in pioglitazone + metformin group, and 1 woman in glimepiride + metformin group), noncompliance (1 man in pioglitazone group and 1 woman in pioglitazone + metformin group), excessive body weight increase (2 women in pioglitazone group), nausea (1 woman in metformin group), gastrointestinal events (1 man and 1 woman in metformin group), and hypoglycemia (FPG <60 mg/dL) (1 man in glimepiride + metformin group).

Of these, 240 patients (118 men and 122 women) completed the study. The reasons for the withdrawal during the last 12 months included being lost to follow-up (1 woman in pioglitazone + metformin group and 1 woman in glimepiride + metformin group), protocol violation (1 woman in pioglitazone group and 1 man in pioglitazone + metformin group), noncompliance (1 man in pioglitazone group), excessive body weight increase (1 woman in pioglitazone group), gastrointestinal events (1 man and 1 woman in metformin group), and hypoglycemia (FPG <60 mg/dL) (2 men in pioglitazone + metformin group and 2 men in glimepiride + metformin group).

3.2. Body mass index

A BMI increase was observed in the groups treated with pioglitazone and with glimepiride + metformin at 15 months

Table 2
Anthropometric and metabolic parameters during the various phases of the study

	Pioglitazone			Metformin			Pioglitazone + metformin			Glimepiride + metformin		
	Baseline	3 mo	15 mo	Baseline	3 mo	15 mo	Baseline	3 mo	15 mo	Baseline	3 mo	15 mo
BMI (kg/m^2)	27.5 ± 1.7	27.7 ± 1.8	28.1 ± 2.0*	27.2 ± 1.5	27.0 ± 1.4	26.7 ± 1.2$^{\#,\dagger\dagger}$	27.4 ± 1.6	27.2 ± 1.5	26.9 ± 1.3$^{\#,\dagger\dagger}$	27.1 ± 1.4	27.8 ± 1.9	28.4 ± 2.2*
HbA_{1c} (%)	9.2 ± 1.3	8.8 ± 1.1*	8.2 ± 0.7†	9.1 ± 1.2	8.6 ± 0.9*	7.9 ± 0.5†	9.3 ± 1.4	8.1 ± 0.6†	7.2 ± 0.3$^{\ddagger,\|,**}$	9.0 ± 1.1	8.5 ± 0.8*	7.8 ± 0.4†
FPG (mg/dL)	164 ± 28	156 ± 23*	151 ± 21†	161 ± 27	153 ± 22†	148 ± 19‡	167 ± 29	150 ± 20‡	139 ± 10$^{\S,\|,**}$	168 ± 30	152 ± 21*	145 ± 16†
PPG (mg/dL)	197 ± 41	192 ± 38	183 ± 33†	192 ± 38	185 ± 34*	171 ± 30$^{\ddagger,\#}$	198 ± 42	181 ± 32†	162 ± 28$^{\S,\|,**}$	199 ± 44	183 ± 33*	169 ± 30$^{\ddagger,\#}$
FPI (μU/mL)	24.8 ± 6.4	23.1 ± 5.9*	21.2 ± 5.6†,††	24.6 ± 6.3	24.2 ± 6.1	22.9 ± 5.8*,††	25.2 ± 6.6	22.6 ± 5.7†	17.8 ± 4.7$^{\ddagger,\|,\ddagger\ddagger}$	23.9 ± 6.0	24.8 ± 6.4	26.2 ± 7.1†
PPI (μU/mL)	79.5 ± 21.7	74.8 ± 20.1*,††	70.6 ± 18.3†,‡‡	81.6 ± 22.2	77.1 ± 21.2*,††	69.8 ± 17.9†,‡‡	84.6 ± 23.5	76.3 ± 20.7†,††	56.4 ± 12.5§,¶,**,§§	84.9 ± 23.9	86.1 ± 24.6	89.8 ± 26.2*
GIR (μmol/[min kg])	5.83 ± 0.68	6.18 ± 0.77*	7.43 ± 0.89$^{\ddagger,\|,\dagger\dagger}$	5.71 ± 0.63	6.02 ± 0.71	6.87 ± 0.81*	5.73 ± 0.64	6.96 ± 0.85†	8.76 ± 0.99$^{\S,\P,\#,\ddagger\ddagger}$	5.64 ± 0.56	5.99 ± 0.69	6.78 ± 0.79*
TGR (μmol/[min kg])	34.8 ± 6.4	36.5 ± 6.7*	40.2 ± 7.5$^{\ddagger,\|,\dagger\dagger}$	34.1 ± 6.2	35.2 ± 6.5	38.1 ± 7.1†	34.6 ± 6.3	37.2 ± 6.9$^{*,\|}$	44.6 ± 8.3$^{\S,\P,\#,\ddagger\ddagger}$	33.2 ± 5.8	34.6 ± 6.3	35.9 ± 6.6*

Data are means ± SD.

* $P < .05$ vs baseline.
† $P < .01$ vs baseline.
‡ $P < .001$ vs baseline.
§ $P < .0001$ vs baseline.
‖ $P < .05$ vs metformin.
¶ $P < .01$ vs metformin.
# $P < .05$ vs pioglitazone.
** $P < .01$ vs pioglitazone.
†† $P < .05$ vs glimepiride + metformin.
‡‡ $P < .01$ vs glimepiride + metformin.
§§ $P < .001$ vs glimepiride + metformin.

($P < .05$ for both groups), whereas no BMI changes were observed in the other 2 groups compared with the baseline. After 15 months, BMI reached in the groups treated with metformin alone and with pioglitazone + metformin was significantly lower than BMI reached in the groups treated with pioglitazone alone or with glimepiride + metformin ($P < .05$ vs pioglitazone and $P < .05$ vs glimepiride + metformin for both groups) (Table 2).

3.3. Glycemic control

In all groups, there was a decrease in HbA_{1c} at 3 and 15 months compared with baseline ($P < .05$ and $P < .01$, respectively, for the groups treated with pioglitazone alone, with metformin alone, and with glimepiride + metformin; $P < .01$ and $P < .001$ for the group treated with pioglitazone + metformin). At 15 months, HbA_{1c} value in the group treated with pioglitazone + metformin was lower compared with that in the group treated with metformin alone ($P < .05$) and with pioglitazone alone ($P < .01$).

There was a decrease in FPG value in all groups at 3 and 15 months compared with the baseline ($P < .05$ and $P < .01$, respectively, for the groups treated with pioglitazone alone and with glimepiride + metformin; $P < .01$ and $P < .001$ for the group treated with metformin alone; $P < .001$ and $P < .0001$ for the group treated with pioglitazone + metformin). After 15 months, FPG value was lower in the group treated with pioglitazone + metformin compared with the groups treated with metformin alone ($P < .05$) and with pioglitazone alone ($P < .01$).

There was a decrease in PPG value after 15 months, but not after 3 months, compared with the baseline in the group treated with pioglitazone ($P < .01$), whereas there was a PPG decrease at 3 and 15 months in the other groups ($P < .05$ and $P < .001$, respectively, in the groups treated with metformin alone and with glimepiride + metformin; $P < .01$ and $P < .0001$, respectively, in the group treated with pioglitazone + metformin). After 15 months, PPG value in the groups treated with metformin and with glimepiride + metformin was lower than the value observed in the group treated with pioglitazone ($P < .05$, respectively, for both groups), whereas PPG value observed was lower in the group treated with pioglitazone + metformin compared with metformin alone ($P < .05$) and with pioglitazone alone ($P < .01$).

After 3 months, FPI value was lower in the groups treated with pioglitazone alone and with pioglitazone + metformin ($P < .05$ and $P < .01$, respectively), whereas there was no change in the other groups compared with the baseline. After 15 months, there was an increase in FPI value in the group treated with glimepiride + metformin ($P < .01$), whereas there was a decrease in FPI value in the other groups compared with the baseline ($P < .05$ in the group treated with metformin alone; $P < .01$ in the group treated with pioglitazone alone; and $P < .001$ in the group treated with pioglitazone + metformin). After 15 months, FPI value was lower in the groups treated with pioglitazone alone and with metformin alone compared with the group treated with glimepiride + metformin ($P < .05$, respectively, for both groups), whereas there was a further decrease in FPI value in the group treated with pioglitazone + metformin compared with metformin alone ($P < .05$) and with glimepiride + metformin ($P < .01$).

After 3 months, there was no change in PPI value in the group treated with glimepiride + metformin, whereas there was a decrease in PPI value in the other groups compared with the baseline ($P < .05$ in the groups treated with pioglitazone alone and with metformin alone; $P < .01$ in the group treated with pioglitazone + metformin). After 15 months, there was an increase in PPI value in the group treated with glimepiride + metformin ($P < .05$) and a decrease in PPI value in the other groups compared with the baseline ($P < .01$ in the groups treated with metformin alone and with pioglitazone alone; $P < .0001$ in the group treated with pioglitazone + metformin). At 3 months, PPI value observed in the groups treated with pioglitazone alone, with metformin alone, and with pioglitazone + metformin was lower than the value observed in the group treated with glimepiride + metformin ($P < .05$, respectively, for all groups). At 15 months, PPI value was lower compared with the glimepiride + metformin group in the groups treated with pioglitazone alone ($P < .01$), with metformin alone ($P < .01$), and with pioglitazone + metformin ($P < .001$). At 15 months, PPI value in the group treated with pioglitazone + metformin was lower compared with that in the group with pioglitazone alone and with metformin alone ($P < .01$ for both groups).

After 3 months, there was an increase in GIR in the groups treated with pioglitazone alone ($P < .05$) and with pioglitazone + metformin ($P < .01$) compared with the baseline, whereas there were no changes in the other groups. After 15 months, there was a GIR increase in all groups compared with the baseline ($P < .05$ in the groups treated with metformin alone and with glimepiride + metformin; $P < .001$ in the group treated with pioglitazone alone; $P < .0001$ in the group treated with pioglitazone + metformin). After 15 months, GIR value observed in the groups treated with pioglitazone alone and with pioglitazone + metformin was higher compared with that in the group treated with metformin alone ($P < .05$ and $P < .01$, respectively) and with glimepiride + metformin ($P < .05$ and $P < .01$, respectively); furthermore, the value obtained in the group treated with pioglitazone + metformin was higher than the value obtained with pioglitazone alone ($P < .05$).

At 3 months, there was an increase in TGR value in the groups treated with pioglitazone alone and with pioglitazone + metformin ($P < .05$, respectively, for both groups), whereas there was no change in the other 2 groups compared with the baseline. After 15 months, there was an increase in TGR value in all groups compared with the baseline ($P < .05$ in the group treated with glimepiride + metformin; $P < .01$ in the group treated with metformin alone; $P < .001$ in the group treated with pioglitazone alone; $P < .0001$ in the group treated with pioglitazone + metformin, respectively). At 3 months, TGR was higher with pioglita-

zone + metformin compared with metformin alone ($P < .05$). At 15 months, TGR was higher in the groups treated with pioglitazone alone and with pioglitazone + metformin compared with metformin alone ($P < .05$ and $P < .01$, respectively) and with glimepiride + metformin ($P < .05$ and $P < .01$, respectively); furthermore, TGR value in the group treated with pioglitazone + metformin was higher than the value observed in the group treated with pioglitazone alone ($P < .05$) (Table 2).

3.4. Correlations

Stepwise multilinear regression analysis was undertaken to establish which anthropometric and metabolic factors could best predict the insulin resistance (GIR) improvement changes. No significant correlations were found between BMI and metabolic parameters in all groups. Significant predictor of change in insulin resistance (GIR) was HbA_{1c} concentration in pioglitazone group ($r = -0.58$, $P < .02$), in metformin group ($r = -0.51$, $P < .05$), in pioglitazone + metformin ($r = -0.67$, $P < .01$), and in glimepiride + metformin ($r = -0.49$, $P < .05$). Other correlation analyses did not indicate various patterns of associations in FPG, PPG, FPI, PPI, and TGR value with any other parameters.

4. Discussion

Insulin sensitivity and insulin secretion are mutually related such that insulin resistance is compensated by increased insulin secretion [21]. A correct judgement of insulin secretion therefore requires validation in relation to the insulin sensitivity in the same subject, especially when comparing drug effects in the setting of clinical trials [22]. Mathematical analysis of the relationship between insulin sensitivity and insulin secretion has revealed a hyperbolic function, such that the product of the 2 variables is constant [23]. This product is usually called the *disposition index*.

In our study, we compared the effect of 4 antidiabetic treatments on basal and postprandial insulin level and on insulin resistance evaluated by the euglycemic hyperinsulinemic clamp. The compared treatments were metformin alone, as the criterion standard of insulin sensitizer; pioglitazone alone, as a relatively new insulin sensitizer; pioglitazone + metformin; and glimepiride + metformin.

After 3 months of treatment, all tested therapies were associated with a significant improvement in HbA_{1c} (from −0.5% to −1%) and FPG (from +8 to +17 mg/dL) when compared with the baseline, but without difference among groups. On the other side, only the metformin-based treatment experienced a similar reduction in PPG (−16.5 mg/dL), whereas only the pioglitazone-based treatments experienced an improvement on insulin resistance–related parameters: FPI (−7% alone, −10% associated with metformin), GIR (+7% alone, +23% associated with metformin), and TGR (+5% alone, +7.5% associated with metformin). The only parameter that differed among the treatment protocol was PPI, which significantly decreased in all groups beyond the one treated with glimepiride, according to the action mechanism of this drug [24].

After 15 months of treatment, all tested therapies were able to significantly improve all the tested glucose- and insulin-related parameters when compared with the baseline. All treatments beyond the glimepiride-based one were combined with a significant reduction in PPI: in particular, PPI decreased by 33% vs baseline in the pioglitazone + metformin–treated group, whereas it increased by +9.6% in the glimepiride + metformin one. The other insulin resistance–related parameters significantly improved in all the groups, but more so in the pioglitazone + metformin group than in the other ones: FPI, −30%; GIR, +52.6%; and TGR, +29% (vs a mean improvement in other groups of −10%, +23%, and +12%, respectively).

After 15 months of treatment, BMI also significantly decreased in the metformin (−1.8%) and pioglitazone + metformin (−1.8%) groups, whereas it increased in the pioglitazone (+2.2%) and glimepiride + metformin (+4.8%) groups.

The effect of different oral antidiabetic treatment with more or less insulin-sensitizing efficacy had already been tested either by our research group [25,26] or by other authors [27,28], and the observed results are in line with previously published reports.

What is relatively new is the parallel evaluation of the insulin-sensitizing activity of different antidiabetic protocols evaluated with the euglycemic hyperinsulinemic clamp in a sufficiently large amount of patients. In fact, this technique has tested the effect of pioglitazone [29], metformin [30], and glimepiride [31] separately, with results similar to that verified in our study, but never together and often in small groups of patients.

Our study also has some limitations. The first one is the lack of intermediate evaluation of clamp measurement between the third and the 15th month of treatment. However, no change in therapy has been observed during the study. Another limitation is the relatively small amount of subjects randomized per tested treatment, which on the other side is higher than that available in most studies where the euglycemic hyperinsulinemic clamp was carried out. One more drawback is that insulin secretion cannot be directly evaluated from the euglycemic clamp. When using the clamp, therefore, it would have been necessary to carry out another experiment, such as a primed hyperglycemic glucose clamp, an intravenous glucose tolerance test, an arginine test, or any other experiment where the β-cell is stimulated (not necessarily only by glucose) to release insulin, which we did not [32]. We limited, in fact, our study of insulin secretion to the evaluation of PPI and its changes under various treatment protocols.

Anyway, to the best of our knowledge, this is the first randomized clinical trial directly comparing the insulin-sensitizing effect of 4 different oral antidiabetic drug regimens.

In conclusion, on the basis of our study carried out on 4 groups of 60 type 2 diabetes mellitus patients monitored by the euglycemic hyperinsulinemic clamp, it appears to confirm that pioglitazone-metformin–based therapeutic control is associated with the most quantitatively relevant improvement in insulin resistance–related parameters, whereas the sulfonylurea-metformin–including protocol has less relevant effects.

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