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Differential regulation of insulin action and tumor necrosis factor α system activity by metformin

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Abstract

Background: Tumor necrosis factor α has a key role in insulin resistance. We study the effects of metformin on glucose tolerance, insulin resistance, beta cell function, and soluble tumor necrosis factor receptor (sTNFR) levels.

Methods: We performed a double-blind, randomized metformin-placebo study. Twenty-three subjects with impaired glucose tolerance or impaired fasting glucose were studied. Oral glucose tolerance, homeostasis model assessment, and continuous infusion of glucose with model assessment tests were used to evaluate glucose tolerance, insulin sensitivity, and beta cell function, respectively. Soluble tumor necrosis factor receptor levels were measured before and after therapy. Repeated measures analysis of variance was used for statistical analysis.

Results: After 12-week treatment, fasting glucose (110.1 ± 9.9 to 98.9 ± 15.7 mg/dl, $P < .001$), fasting insulin (11.6 ± 5.4 to 8.8 ± 3.5 mU/L, $P = .05$), fasting C-peptide (2.5 ± 0.7 to 1.8 ± 0.5 ng/mL, $P < .05$), and achieved C-peptide (5.2 ± 1.2 to 4.2 ± 1 ng/mL, $P < .05$) levels decreased in the metformin group. In addition, there was an improvement in insulin sensitivity (37.4% ± 15.2% to 50.4% ± 23.2%, $P < .05$) with unchanged sTNFR1 (2.0 ± 0.8 to 2.3 ± 1.2 μg/L, P = NS) and sTNFR2 (4.8 ± 1.7 to 4.4 ± 1.2 μg/L, P = NS) levels.

Conclusions: Metformin is able to reverse insulin resistance and hyperglycemia in high-risk subjects for type 2 diabetes mellitus independently of the effects on tumor necrosis factor α system activity.

1. Introduction

Impaired glucose tolerance (IGT) and abnormal fasting glucose (IFG) should be considered major risk factors for type 2 diabetes and important risk markers for cardiovascular disease [1-3]. Annual conversion rates to frank type 2 diabetes mellitus depend on a number of factors such us ethnic group, family history, body mass index (BMI), and exercise and can vary between 1.5% and 7.3% [4,5]. These subjects manifest early defects in insulin resistance and beta cell function [6-9]. Lifestyle intervention [10,11], insulin sensitizers such as metformin [12] and troglitazone [13], and acarbose [14] have been shown to reduce the incidence of type 2 diabetes in high-risk individuals.

Metformin is an insulin-sensitizing biguanide whose glucose-lowering effects are not well understood. It acts primarily by inhibiting gluconeogenesis and, to a lesser extent, glycogenolysis in the liver and by increasing insulin-stimulated glucose uptake in muscle and adipocytes [15]. Recently, increased phosphorylation and activation of adenosine monophosphate–activated protein kinase have been described as the main mechanisms of action of metformin [16].

Tumor necrosis factor α (TNF-α), an inflammatory mediator, has a key role in mediating insulin resistance through the decrease in tyrosine kinase activity of the insulin receptor. TNF-α binds to 2 TNF-α receptors, TNFR1 and TNFR2. Each receptor is expressed by most cells and can be regulated independently. After binding to its receptors, a proteolytic cleavage of the extracellular parts elicits the soluble forms, namely, soluble tumor necrosis factor receptor 1 (sTNFR1) (55 kDa) and sTNFR2 (75 kDa) [17,18]. TNF-α values do not usually give precise information about its action. In contrast, sTNFRs are more stable proteins, remain elevated for longer periods, and have been

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validated as sensitive indicators of TNF-α system activation [19]. Circulating sTNFRs were consistently found in patients with sepsis, representing the inflammatory state, even when TNF-α activity was undetectable [20]. Measurements of sTNFR concentrations in healthy individuals at different time lapses showed that levels in the same subject were quite stable over time [21].

We have previously described that plasma sTNFR2 levels were associated with insulin resistance in nondiabetic subjects [22]. Metformin has been able to reverse insulin resistance induced by TNF-α in liver cells [23] and can reverse hepatic steatosis in obese leptin-deficient mice, suggesting an inhibition of liver expression of TNF-α [24]. Regular physical exercise led to a consistent decrease in circulating sTNFR2 in obese women and in type 2 diabetic patients, in parallel with an improvement in insulin sensitivity [25-27].

We hypothesized that metformin might decrease sTNFR levels. Therefore, we performed a double-blind, randomized, placebo-controlled study measuring the effects of metformin on glucose tolerance, insulin resistance, beta cell function, and sTNFR levels in subjects with IGT or IFG.

2. Patients and methods

Patients were recruited from the Diabetes and Endocrinology Unit at Hospital de Girona Dr Josep Trueta. They were offered participation in the study if they were between 30 and 65 years old and fulfilled all the inclusion criteria. Both men and women were included, but women only when surgical sterility was documented or when they were postmenopausal or when a reliable method of contraception was used. The inclusion criteria were as follows: (*a*) BMI between 22 and 35 kg/m^2, (*b*) IGT by an oral glucose tolerance test (OGTT) performed 2 months before the beginning of the study or a fasting glucose level between 110 and 140 mg/dL, (*c*) stability of diet and physical exercise within the past 2 months. All subjects signed a written informed consent. Exclusion criteria were as follows: (*a*) diabetic patients according to the National Diabetes Data Group criteria of 1979 [28], (*b*) pregnant and nursing women, (*c*) patients with renal impairment defined as plasma creatinine values at or above 1.5 mg/dL for men and 1.4 mg/dL for women, (*d*) patients affected by cardiac or respiratory insufficiency likely to cause central hypoxia or reduced peripheral perfusion, (*e*) past history of lactic acidosis, (*f*) noncontrolled hypertension, (*g*) acute or chronic infection, (*h*) liver disease, including alcoholic liver disease as demonstrated by abnormal results on liver function tests or alcohol abuse, (*i*) patients taking drugs that could modify glucose tolerance, (*j*) participants in another clinical trial within the last 30 days, and (*k*) legal incapacity as a study patient.

The clinical study was performed in accordance with the Declaration of Helsinki (revised version of Hong Kong 1989) as well as with the EC Note for Guidance, "Good Clinical Practice for Studies on Medicinal Products in the European Community." It was approved by the local Ethics Committee and the Spanish Health Department (clinical assay number 97/337).

Thirty-one patients were randomized either to placebo or metformin according to the randomization tablets supply generated by LIPHA using the computer program RANCODE +3.1. A dietician gave general diabetic dietary advice at the beginning of the study to ensure that the subjects had a stable weight. Two months after the first visit (visit 2), subjects started taking metformin or placebo, 1 tablet per day (850 mg) for the first week and then 2 tablets per day (1 after breakfast and 1 after dinner) for the following 11 weeks. Drug compliance was checked by tablet counts, and any side effect was recorded at visit 3 (6 weeks after randomization) and at visit 4 (at the end of the study). Additional concomitant therapy was also noted. Weight, BMI, waist to hip ratio, and blood pressure after 5 minutes rest (BP-103N-Mark III sphygmomanometer, Nippon Colin Co Ltd, Komaki, Japan) were measured in all 4 visits. Blood tests were performed before randomization and at the end of treatment, including fasting glucose (photometry method, Hitachi modular autoanalyzer P800, Roche Diagnostics, Basel, Switzerland) and 24-hour urine albumin (immunoturbimetry method). To assess glucose tolerance, an OGTT was performed after an overnight fast. Insulin sensitivity was evaluated by homeostasis model assessment (HOMA) using basal (mean of 3 samples obtained at 5-minute intervals) glucose and insulin [29,30]. Beta cell function was calculated by continuous infusion of glucose with model assessment (CIGMA) from achieved C-peptide and glucose values [31]. CIGMA test consists of a continuous intravenous infusion of 5 mg glucose per kilogram of ideal body weight per minute using a 10 g/dL glucose solution with model assessment of glucose, insulin (IRMA, Medgenix Diagnostics, Fleurus, Belgium), and C-peptide (RIA, Byk-Sangtec Diagnostica, Dietzenbach, Germany) values obtained before (basal value, mean value of 3 samples obtained at 5-minute intervals) and at the end (achieved value, mean value of samples obtained at 50, 55, and 60 minutes) of the test. Insulin detection level was 4 mU/L with an intra-assay and interassay coefficient of variation of 5.2% and 6.9%, respectively, at 10 mU/L concentration and of 3.4% and 4.5%, respectively, at 130 mU/L concentration. There was no cross-reactivity with proinsulin or C-peptide. C-peptide detection level was 0.1 ng/mL and had intra- and interassay coefficients of variation of 2.6% and 4.4%. It shows 25% cross-reactivity with proinsulin but not with insulin.

Plasma sTNFR1 and sTNFR2 levels were analyzed by commercially available solid phase enzyme-amplified sensitivity immunoassays (EASIA), Medgenix sTNFR1 and sTNFR2 EASIA (BioSource Europe SA, Zoning Industriel B-6220, Fleunes, Belgium). The minimum detectable concentration was estimated to be 0.1 ng/mL and was defined as the sTNFR1 or sTNFR2 concentration corresponding to the

Table 1
Baseline characteristics of the analyzed patients

	Metformin (n = 11)	Placebo (n= 12)	*P*
Age (y)	46.7 ± 7.8	46.5 ± 6.7	NS
BMI (kg/m^2)	28.0 ± 4.5	28.8 ± 4.0	NS
Sex (male/female)	5/6	5/7	NS
Family history of diabetes	6/5	5/7	NS
IGT/IFG	9/2	7/5	NS

Data are mean ± SD.

average optical density (OD) of 20 replicates of the zero standard + 2 standard deviations. Intra- and interassay coefficients of variation were <7% and <9%. sTNFR1 EASIA did not cross-react with sTNFR2. TNF-α did not interfere with the assay.

2.1. Statistical analysis

To detect differences in achieved glucose more than 18 mg/dL, we estimated a sample size of 15 patients in each group, considering 10% of losses. The α and β error was .05 and .1, respectively, in a 1-sided test. Continuous variables were expressed as mean and SD and compared by the Student *t* test or the Mann-Whitney *U* test as appropriate. Comparisons of proportions between groups were made using the χ^2 test. Analysis of the end points between groups was made by means of a generalized linear model (analysis of variance) for repeated measurements. Significance level was 5% and the statistical program used the SPSS 10.0 (1999 SPSS Inc, Chicago, Ill).

3. Results

Out of 118 preselected subjects, 31 patients were randomized for the study (16 to metformin and 15 to placebo). Six patients (3 on metformin and 3 on placebo) discontinued early and 2 noncompliant patients were excluded (both in the metformin group). We finally analyzed 23 patients (11 on metformin and 12 on placebo).

Table 1 shows the baseline characteristics of the analyzed patients. They were of similar age, BMI ,and sex. Fasting glucose, insulin, and C-peptide levels were similar (Table 2). Insulin sensitivity, beta cell function, and sTNFRs levels were also equal (Table 3).

After 12-week treatment, fasting glucose decreased in the metformin group (metformin [M], 110.1 ± 9.9 to 98.9 ± 15.7 mg/dL; placebo [P], 106.9 ± 8.9 to 106.3 ± 6.5 mg/dL, *P* = .004) (Table 2). Fasting insulin (M, 11.6 ± 5.4 to 8.8 ± 3.5 mU/L; P, 11.2 ± 3.3 to 11.5 ± 3.3 mU/L, *P* = .05), fasting C-peptide (M, 2.5 ± 0.7 to 1.8 ± 0.5 ng/mL; P, 2.1 ± 0.7 to 2.0 ± 0.8 ng/mL, *P* = .02), and achieved C-peptide (M, 5.2 ± 1.2 to 4.2 ± 1 ng/mL; P, 4.4 ± 1.4 to 4.5 ± 1.5 ng/mL, *P* = .02) levels were also statistically lower after metformin treatment (Table 2). Insulin sensitivity by HOMA (M, 37.4% ± 15.2% to 50.4% ± 23.2%; P, 35.4% ± 10.1% to 34.6% ± 9.9%, *P* = .02) improved after metformin treatment and was not modify by placebo. Beta cell function was not modified in either group (Table 3). sTNFR1 (M, 2.0 ± 0.8 to 2.3 ± 1.2 μg/L; P, 1.7 ± 0.4 to 2.2 ± 1.0 μg/L, *P* = .6) and sTNFR2 (M, 4.8 ± 1.7 to 4.4 ± 1.2 μg/L; and P, 5.1 ± 1.9 to 4.8 ± 2.0 μg/L, *P* = .8)

Table 2
Clinical and metabolic characteristics of the patients before and after treatment

	Metformin		Placebo	
	Baseline	12 wk	Baseline	12 wk
Clinical characteristics				
BMI (kg/m^2)	28.0 ± 4.5	27.7 ± 2.3	28.8 ± 4.0	28.4 ± 3.8
Waist circumference	95.9 ± 11.5	94.8 ± 9.4	95.5 ± 10.4	93.9 ± 10.6
Waist/hip ratio	0.96 ± 0.1	0.92 ± 0.07	0.91 ± 0.07	0.92 ± 0.07
OGTT				
Glucose 120 (mg/dL)	162.2 ± 27.8	140.6 ± 42.9	130.3 ± 31.0	139.5 ± 28.9
HOMA/CIGMA				
Fasting glucose (mg/dL)	110.1 ± 9.9	98.9 ± 15.7*	106.9 ± 8.9	106.3 ± 6.5
Achieved glucose (mg/dL)	190.5 ± 18.9	174.6 ± 34.5	194.8 ± 17.1	180.9 ± 13.9
Fasting insulin (mU/L)	11.6 ± 5.4	8.8 ± 3.5**	11.2 ± 3.3	11.5 ± 3.3
Achieved insulin (mU/L)	28.5 ± 13.5	23.3 ± 7.6	27.7 ± 11.3	31.3 ± 14.2
Fasting C-peptide (ng/mL)	2.5 ± 0.7	1.8 ± 0.5**	2.1 ± 0.7	2.0 ± 0.8
Achieved C-peptide (ng/mL)	5.2 ± 1.2	4.2 ± 1.0**	4.4 ± 1.4	4.5 ± 1.5

Data are means ± SD.
* *P* < .01.
** *P* < .05 (within group).

Table 3
Insulin sensitivity, beta cell function, and sTNFR values before and after treatment

	Metformin		Placebo	
	Baseline	12 wk	Baseline	12 wk
HOMA sensitivity (%)	37.4 ± 15.2	50.4 ± 23.2*	35.4 ± 10.1	34.6 ± 9.9
CIGMA beta cell function (%)	82.8 ± 24.0	90.9 ± 34.0	71.7 ± 27.2	83.1 ± 30.0
sTNFR1 (μg/L)	2.0 ± 0.8	2.3 ± 1.2	1.7 ± 0.4	2.2 ± 1.0
sTNFR2 (μg/L)	4.8 ± 1.7	4.4 ± 1.2	5.1 ± 1.9	4.8 ± 2.0

Data are mean ± SD.
* $P < .05$ (inside group).

levels were not modified after metformin therapy or placebo (Table 3).

No differences were observed in BMI or waist to hip ratio during the study (Table 2). Only 4 patients (17.4%) described adverse effects: 2 patients reported diarrhea (8.7%, both in the metformin group) and 2 patients complained of heartburn and nausea (8.7%, both in the placebo group). Adverse effects were not statistically different between groups ($P = .2$).

4. Discussion

Metformin has been used as an oral treatment of type 2 diabetes for the past 40 years. It has various mechanisms of action and some of them remain unclear. Its primary function is to improve insulin sensitivity in the liver, decreasing hepatic glucose production mainly by inhibition of the glucose-6 phosphatase activity, and promoting glycogen sparing [32]. It also increases glucose disposal in skeletal muscle and might decrease intestinal absorption of glucose [15]. It has been suggested that metformin metabolic effects may be mediated by the activation of adenosine monophosphate–activated protein kinase in cultured rat hepatocytes and in subjects with type 2 diabetes [16,33].

Tumor necrosis factor α might be involved in modulating insulin action in subjects at high risk for type 2 diabetes [34]. First-degree relatives of subjects with type 2 diabetes mellitus showed high levels of sTNFR2, suggesting that the presence of abnormalities in the TNF-α pathway could predispose to the development of the disease [35]. Metformin seems to inhibit hepatic TNF-α expression and is able to reverse several TNF-α–inducible responses that promote hepatic steatosis [24].

Our study shows new data regarding the ability of metformin to decrease insulin resistance and improve hyperglycemia in high-risk prediabetic subjects. The Diabetes Prevention Program study demonstrated that metformin reduced the incidence of diabetes by 31%, although lifestyle intervention was more effective, reducing it by 58% [12]. Other studies have shown that metformin was superior to placebo in decreasing the conversion rate of subjects with IGT to diabetes [36,37]. In the Diabetes Prevention Program, metformin effects were greater mainly in overweight subjects (BMI > 35); all our patients had BMI less than 35 kg/m^2, and metformin was also effective, although our objective was the study of metabolic effects in a short period and not the prevention of diabetes.

We found that metformin was more effective in reducing fasting glucose levels than glucose levels under TTOG or CIGMA test, reflecting its main action in the liver of reducing gluconeogenesis and hepatic insulin resistance. Probably, patients with IFG should benefit more from the effect of metformin than do patients with IGT. Larger studies will be necessary to detect these differences.

We could not find differences in sTNFRs, suggesting that metformin effects are not mediated by changes in TNF-α action, as other studies performed in nondiabetic patients have suggested [38]. Altered glucose tolerance and the insulin resistance syndrome lead to an ongoing acute-phase response through increased cytokines derived from unsuppressed adipose tissue [39]. When proinflammation is enduring, chronic or uncontrolled improvement of insulin resistance by pharmacologic means could not be sufficient, in quantity or quality, to reverse this proinflammatory phenotype [40]. However, insulin resistance could be the result of different altered mechanisms and could vary between individuals. We found that metformin improved insulin sensitivity in these individuals by a mechanism not related to TNF-α. It seems that TNF-α may modulate insulin receptor signaling in skeletal muscle and liver through different pathways. TNF-α decreases insulin action in muscles by down-regulation of insulin receptor autophosphorylation. Its action in the liver, where metformin exerts its main function, has to be well elucidated. It probably acts downstream from the insulin receptor modifying gluconeogenic substrates [41].

We can conclude that metformin is able to reverse insulin resistance and hyperglycemia in high-risk subjects for type 2 diabetes mellitus independently of the effects on TNF-α system activity.

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