Blood Pressure and Cardiac Autonomic Nervous System in Obese Type 2 Diabetic Patients: Effect of Metformin Administration

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Background: Hyperinsulinemia/insulin resistance and elevated plasma free fatty acids (FFA) levels are involved in the hypertension and cardiac sympathetic overactivity. Metformin improves insulin action and lower plasma FFA concentrations. We investigate the possible effect of metformin on arterial blood pressure (BP) and cardiac sympathetic nervous system.

Methods: One hundred twenty overweight type 2 diabetic patients were treated by placebo ($n = 60$) + diet or metformin (850 mg twice daily) ($n = 60$) + diet for 4 months, to evaluate the effect of metformin treatment on the cardiac autonomic nervous system. Insulin resistance was measured by the Homeostasis Model Assessment (HOMA) index. Heart rate variability (HRV) assessed cardiac sympathovagal balance.

Results: Metformin treatment, but not placebo treatment, was associated with a decrease in fasting plasma glucose ($P < .05$), insulin ($P < .05$), triglyceride ($P < .05$), and FFA ($P < .03$) concentrations and HOMA index ($P < .03$). Metformin treatment was also associated with a significant improvement in cardiac sympathovagal balance but not in mean arterial BP. Furthermore, in a multivariate analysis, delta change in sympathovagal balance index (LF/HF ratio) were associated with delta change in plasma FFA concentrations and HOMA index independently of gender and delta change in plasma triglyceride and HbA1c concentrations.

Conclusions: Our study demonstrated that metformin treatment might be useful for improving cardiac sympathovagal balance in obese type 2 diabetic patients. Am J Hypertens 2004;17:223–227 © 2004 American Journal of Hypertension, Ltd.

Key Words: Metformin, free fatty acids, heart rate variability, insulin resistance, blood pressure.

It is widely accepted that hyperinsulinemia/insulin resistance increases cardiovascular mortality in type 2 diabetic patients due to sympathetic overactivity.[1,2] Some recent data have also provided evidence that elevated plasma-free fatty acid (FFA) concentration causes a stimulation of the cardiac autonomic nervous system and thus also has a proarrhythmic role.[3,4] Because metformin improves insulin action and lowers plasma FFA concentrations one cannot rule out that metformin could also affect the cardiac autonomic nervous system.

On the other hand, a direct effect of metformin on sympathetic nervous activity has been demonstrated by the ability of the drug to interrupt neurotransmission in sympathetic ganglia.[5] Thus, it is possible to hypothesize that chronic metformin treatment in insulin resistant type 2 diabetic patients might be associated with a lowering of cardiac sympathetic tone and with a positive modulation of the sympathovagal balance by an indirect (decline in plasma FFA and or in insulin resistance) and by a direct effect on the cardiac autonomic nervous system.

To the best of our knowledge no study has addressed such a possibility in humans. Accordingly, we investigated the possible effect of metformin versus placebo treatment on cardiac autonomic nervous activity, assessed by heart rate variability (HRV),[6] in 120 overweight type 2 diabetic patients (64 men/56 women).

Methods

One hundred twenty overweight type 2 diabetes mellitus outpatients in treatment with only diet volunteered for the study. All patients had no evidence of coronary heart

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Table 1. Clinical characteristics of study groups at baseline and after treatment with placebo (n = 60) or metformin (n = 60)

	Baseline	*P*	Placebo	Baseline	*P*	Metformin
Gender (M/F)	33/27			31/29		
BMI (kg/m^2)	29.2 ± 0.2	NS	29.5 ± 0.4	29.5 ± 0.1	NS	29.1 ± 0.2
WHR	0.92 ± 0.35	NS	0.92 ± 0.26	0.94 ± 0.31	NS	0.89 ± 0.14
Glucose (mmol/mL)	8.3 ± 0.9	NS	8.2 ± 0.3	8.2 ± 0.5	.05	7.3 ± 0.2
Insulin (pmol/mL)	83.4 ± 2.9	NS	83.2 ± 1.5	82.4 ± 2.1	.05	66.1 ± 1.3†
Triglycerides (mmol/L)	2.27 ± 0.2	NS	2.20 ± 0.11	2.22 ± 0.4	.05	1.94 ± 0.15*
Free fatty acids (mmol/L)	660 ± 49	NS	661 ± 57	664 ± 45	.03	556 ± 42†
HbA1c (%)	8.1 ± 0.2	NS	7.9 ± 0.3	8.0 ± 0.2	.05	7.2 ± 0.1*
HOMA index	3.97 ± 0.15	NS	3.95 ± 0.12	4.02 ± 0.14	.03	3.01 ± 0.11†

All results are mean ± SD.
BMI = body mass index; WHR = waist hip ratio.
All parameters are considered at fasting condition.
Homeostasis assessment model (HOMA). No difference were found between two groups at baseline condition.
Placebo versus metformin: * $P < .05$; † $P < .03$.

disease as confirmed by electrocardiogram, echocardiography, and treadmill test. Furthermore, all patients underwent Ewing tests[7] to exclude the occurrence of diabetic neuropathy. More detailed characteristics of patients are given in Table 1. After clear explanation of potential risks of the study, each volunteer gave written informed consent to participate in the study, which was approved by the Ethical Committee of our Institution.

Study Protocol

The study was designed as a randomized parallel group trial of placebo + diet versus metformin + diet. At baseline all patients were studied at 8:00 AM, in a quiet comfortable room at a temperature ranging between 22° and 24°C, after an overnight fast (at least 12 h). A venous blood sample for plasma metabolites was immediately drawn. Then, each patient rested in the supine position for at least 30 min before starting baseline Holter recording, which lasted 60 min. Then, all patients were randomly assigned to placebo (n = 60) or metformin treatment at a dose of 850 mg twice daily (Metforal, Guidotti, Italy; n = 60). Each treatment lasted 4 months. At the end of this treatment period, a complete reevaluation of the patients was made.

Diet

All patients consumed a weight-stable diet (±1500 kcal) of carbohydrate (~50%), fat (~25%), and protein (~25%). The polyunsaturated-to-saturated fatty acids ratio was 1.0. The amount of fiber in the diet was ~10 g/d. The patients were encouraged not to eat additional foods.

Anthropometric Determinations

Weight and height were measured using a standard technique. Body mass index (BMI) was calculated as body weight (in kilograms)/height (meters squared). Waist circumference was measured at the midpoint between the lower rib margin and the iliac crest (normally the umbilical level), and hip circumference was measured at the trocanter level. Both circumferences were measured at the nearest 0.5 cm with plastic tape and the ratio between them provided the waist/hip ratio. The anthropometric measurements are used because the change in body composition may significantly affect both insulin resistance and cardiac autonomic nervous activity. Thus, because our study lasted 4 months, one could also hypothesize that changes in HRV parameters and insulin resistance degree might depend on the changes in body composition.

HOMA Index

Insulin resistance was assessed by the Homeostasis Model Assessment (HOMA).[8] HOMA is a mathematical model describing the degree of insulin resistance starting from patient's fasting plasma insulin and glucose concentrations.[8] The accuracy and precision of the HOMA method have been compared with independent estimates of insulin resistance.[8]

Data Acquisition and Analysis

The software used for data acquisition and analysis has been previously described.[9,10] In brief, the computer program first calculates the interval thacogram. From a section of thacogram of 512 interval values, simple statistics (mean and variance) are calculated. The computer program automatically calculates the autoregressive coefficients necessary to define the power spectral density estimate and prints out the power and frequency of each spectral component. Two major oscillatory components are usually detectable: 1) HF (~0.25 Hz and varying with respiration) is synchronous with respiration; 2) corresponding to the slow waves of arterial pressure, LF (~0.1 Hz). Each spectral component is presented in normalized form (normalized units [nu]), by dividing it by the total power minus the direct current component, if present. Only components >5% of total power were considered significant. Respiratory frequency was also calculated over

Table 2. Cardiovascular parameters of study groups at baseline and after treatment with placebo (n = 60) or metformin (n = 60)

	Baseline	*P*	Placebo	Baseline	*P*	Metformin
MABP (mm Hg)	110 ± 1.6	NS	109 ± 1.2	112 ± 1.8	NS	108 ± 2.9
RR interval (msec)	759 ± 12	NS	755 ± 10	758 ± 10	.05	866 ± 12*
Total power (msec^2)	2744 ± 296	NS	2726 ± 312	2711 ± 395	.05	2915 ± 348*
LF (nu)	70.1 ± 1.6	NS	67.6 ± 1.3	68.6 ± 1.5	.05	52.1 ± 1.1*
HF (nu)	22.5 ± 1.6	NS	22.1 ± 1.8	22.4 ± 1.8	.05	23.6 ± 2.1*
LF/HF ratio	4.8 ± 0.2	NS	4.5 ± 0.3	4.7 ± 0.3	.02	2.9 ± 0.2†

All results are mean ± SD.
MABP = mean arterial blood pressure; LF = low frequency; HF = high frequency.
No differences were found between the two groups at baseline condition. Placebo versus metformin: * $P < .05$; † $P < .02$.

a period of 2 min before the test. Subjects with a respiratory rate less than 10 breaths/min (ie, <0.15 Hz) were excluded from the study. The LF/HF ratio is considered an index of cardiac sympathetic/parasympathetic tone balance.[6,11,12]

Analytical Techniques

Plasma glucose concentrations were determined by the glucose oxidative methods (glucose autoanalyzer, Beckman Coulter, Inc., Fullerton, CA). Plasma insulin concentrations were determined by radioimmunoassay (Linco Research, Inc., St. Charles, MO). Plasma fasting triglyceride concentrations were determined by routine laboratory methods. Plasma fasting FFA concentrations were measured according to Miles et al.[13] Stable HbA1 levels were determined in triplicate according to Compagnucci et al[14] by ion-exchange microcolumns at constant temperature (18°C).

Statistical Analyses

All results are mean ± SD. Mean arterial blood pressure (BP) was calculated as diastolic BP plus one-third pulse pressure. Changes in HbA1c, triglycerides, HOMA index, and LF/HF ratio are used only to compare the changes (and not just the absolute values) of these variables. Because of the skewed distribution, total power, LF, HF, plasma insulin, triglyceride, FFA concentrations, and HOMA index were logarithmically transformed for statistical testing and back transformed for presentation in table. Analysis of variance (ANOVA) allowed calculating difference between the two study groups. Multivariate regression analysis tested the independent association and contribution of gender, Δ changes in HOMA index, plasma FFA, triglyceride, and HbA1c levels with the dependent variable (LF/HF ratio). A P value of .05 was chosen as the level of significance. All calculations were made on an IBM personal computer by SPSS 10.0 (SPSS, Chicago, IL).

Results

All volunteers completed the study. All patients were adults (age, 57±11 years) and nonsmokers (Table 1). At baseline anthropometric and metabolic parameters were similar in both study groups. At the end of study, metformin, but not placebo administration, was associated with a significant decrease in fasting plasma glucose, insulin, triglyceride, FFA, and HbA1c concentrations, as well as in insulin resistance (HOMA) (Table 1). With regard to cardiovascular parameters (Table 2), both groups had similar values at baseline. Mean arterial BP was unaffected by both treatment modes. In contrast, metformin versus placebo, had a strong impact on HRV parameters. In fact, an increase in RR interval, total power, and HF, and a decrease in LF and LF/HF ratio were found after metformin administration (Table 2).

Due to the occurrence of statistically significant differences in plasma HbA1c, triglyceride, and FFA concentrations, HOMA index and LF/HF ratio before and after metformin treatment, delta (Δ)μ changes in these parameters were calculated. Thus, a multivariate analysis was made using these parameters. In such analysis LF/HF ratio was the dependent variable, whereas gender, Δ changes in HOMA index, plasma FFA, triglyceride, and HbA1c levels were the independent variables. Such a model explained 73% of the variability in the Δ changes in the LF/HF ratio with Δ changes in plasma FFA level (t = 2.65; $P < .01$) and HOMA index (t = 2.42; $P < .03$) independently and significantly associated with Δ changes in the LF/HF ratio. Furthermore, percentage changes in plasma FFA and HOMA index explained 26% and 18% of the LF/HF ratio variability, respectively. Only 5 patients had minor gastrointestinal symptoms, such as side effects of metformin administration.

Discussion

Our results demonstrate that the metformin-related decrease in plasma FFA and insulin resistance is associated with an improvement in cardiac autonomic nervous balance in overweight type 2 diabetic patients.

Type 2 diabetic patients are characterized by a greater increased cardiac mortality than healthy subjects.[15] Among the factors responsible for such elevated mortality, hyperinsulinemia/insulin resistance and high plasma FFA concentrations are considered to play a pivotal role

through an increase in sympathetic activity.[4,16–18] Nevertheless, the underlying mechanisms responsible for such sympathetic overactivity are not fully understood. In humans, the relationship between hyperinsulinemia and sympathetic activity seems strengthened by measurements of plasma cathecolamine concentrations,[19] plasma norepinephrine spillover,[20] direct microneurographic recordings of sympathetic nerve action,[21] and also by evaluation of cardiac autonomic activity studied by HRV technique.[9,22] In particular, it has been demonstrated that acute infusion of insulin is associated with a significant increase in cardiac sympathetic activity in healthy subjects[9] and in patients affected by insulin resistance.[22] An impact of insulin on the central nervous system has been hypothesized, as insulin crosses the blood–brain barrier, and the insulin receptor has been demonstrated in several distinct regions of the central nervous system such as the median hypothalamus.[23]

The relationship between plasma FFA concentrations and the autonomic nervous system was suggested by Bulow et al[24] showing a vasoconstriction secondary to local perfusion of adipose tissue with FFA. Later, Stepniakowski et al[25] reported that infusion of lipid emulsion plus heparin reduced vein distensibility in healthy volunteers and increased responsiveness to phenylephrine. Grekin et al[26] reported that portal FFA infusion also has significant pressor effects, which may be mediated by increased sympathetic tone. We also showed that elevated plasma FFA concentrations per se might stimulate the cardiac sympathetic nervous system in healthy subjects[4] and in type 2 diabetic patients.[17] Recently we demonstrated that increased postprandial FFA concentrations are associated with an increase in oxidative stress and a neural pressor response, which have a negative impact on sympathetic and parasympathetic balance.[18]

The effect of metformin on insulin resistance[27] and plasma FFA concentrations[28] has been experimentally supported. In particular, Abbasi et al[28] have demonstrated that FFA concentrations were lower after metformin treatment, and suggested that metformin can act on the adipose tissue decreasing FFA release, which, in turn, results in a decline of circulating FFA concentrations. These events should enhance muscle glucose disposal. Due to the strong relationship occurring between insulin resistance/hyperinsulinemia and sympathetic nervous system overactivity, metformin administration could bring down the sympathetic overactivity observed in our patients.

To the best of our knowledge we are the first to evaluate such an effect of metformin on cardiac autonomic nervous activities, which seems related to a decrease in plasma FFA concentrations and insulin resistance (HOMA). In fact, in our study, the multivariate analysis showed that changes in plasma FFA concentration and in HOMA index were associated with a change in LF/HF ratio independently of gender and of the changes in plasma triglyceride and HbA1c levels.

It should be pointed out that an additional effect of metformin might be due to a direct effect on the sympathetic nervous activity. Actually, this effect has been demonstrated only in an experimental model. In particular, Santure et al[29] suggested that, in spontaneous hypertensive rats, part of the beneficial effect of metformin on insulin resistance results from a potentiation of the hormone-stimulating effect on glucose transport in peripheral tissues. Petersen and Di Bona[30] have shown that metformin decreases BP, heart rate, and efferent renal sympathetic nerve activity after intracerebroventricular administration. Thus, they concluded that metformin has acute sympathoinibitory effects produced by a direct central system site of action. Unfortunately, our study design was not appropriate to investigate the potential effect of metformin per se on arterial BP and cardiac autonomic nervous system and thus, we cannot rule out or confirm such an effect on humans. Nevertheless, even if there is a limitation in our study with regard to HRV on sympathetic cardiovascular control, there is great interest in investigating the sympathovagal balance alteration, especially in relation to the unexpected sudden deaths, in diabetic patients. Thus, a HRV test seem to be an appropriate test to be used in our study.

In conclusion, our study demonstrated that metformin-related changes in plasma FFA and in insulin resistance may be useful to improve autonomic nervous system balance at the cardiac level but not arterial BP in overweight type 2 diabetic patients.

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