CLINICAL SCIENCE

SHORT-TERM TREATMENT WITH METFORMIN IMPROVES THE CARDIOVASCULAR RISK PROFILE IN FIRST-DEGREE RELATIVES OF SUBJECTS WITH TYPE 2 DIABETES MELLITUS WHO HAVE A METABOLIC SYNDROME AND NORMAL GLUCOSE TOLERANCE WITHOUT CHANGES IN C-REACTIVE PROTEIN OR FIBRINOGEN

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OBJECTIVE: To study if metformin, when administered to first-degree relatives of type 2 diabetes mellitus subjects who have metabolic syndrome and normal glucose tolerance, could improve the cardiovascular risk profile and reduce the levels of both C-reactive protein and fibrinogen.

INTRODUCTION: Metabolic syndrome is associated with higher cardiovascular morbidity and mortality. Metformin has vasculo-protective effects even in normoglycemic subjects, and C-reactive protein and fibrinogen are considered markers of endothelial injury and inflammation.

METHODS: Thirty-one non-diabetic first-degree relatives of type 2 diabetes mellitus subjects with metabolic syndrome were randomized (1:1) and double-blinded for placement in the placebo and metformin groups (850mg bid/±90days); 16 subjects were administered metformin (mean age 40.0 [33.5-50] years; 13 females) and 15 subjects were in the placebo group (mean age 37.0 [32-42] years; 9 females). Blood samples were collected at baseline and at the end of treatment for biochemical analyses, including an assessment of C-reactive protein and fibrinogen levels.

RESULTS: Metformin improved the lipid profile and decreased fasting plasma glucose, systolic blood pressure, weight and body mass index without changing body composition. For those in the placebo we identified no changes in fibrinogen (282.2 [220.4-323.7] mg/L vs. 286.7 [249.6-295.1] mg/L; NS) or in C-reactive protein levels (0.68 [0.3-1.2] vs. 0.64 [0.3-1.0] mg/L; NS). The same was also observed for the levels of fibrinogen (303.9 [217.6-347.6] mg/L vs. 290.9 [251.5-301.9] mg/L; NS) and C-reactive proteins (0.78 [0.3-1.1] vs. 0.80 [0.4-0.9] mg/L; NS) in the metformin group.

CONCLUSIONS: Metformin treatment in first-degree relatives of type 2 diabetes mellitus sufferers who have metabolic syndrome and normal glucose tolerance improved the cardiovascular risk profile without changing the levels of C-reactive protein and fibrinogen.

KEYWORDS: Metformin; Fibrinogen; C-reactive protein; Normoglycemia; Metabolic syndrome.

INTRODUCTION

Metabolic syndrome (MS) is a pre-diabetic state that

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embodies factors implicated in cardiovascular (CV) risk, including dyslipidemia, hypertension, impaired glucose tolerance (IGT) or type 2 diabetes mellitus (T2DM), insulin resistance (IR) and abdominal obesity.[1] Among these, abdominal obesity is the major component and possibly even the pathophysiological cornerstone of CV disease. Excessive accumulation of visceral adipose tissue directly increases CV morbidity and mortality.[2] There is a clear link between markers of inflammation, MS, and atherosclerosis.[2]

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Acute phase proteins are markers of inflammatory systemic response, and their plasma concentrations increase or decrease by at least 25 percent during an inflammatory challenge. C- reactive protein (CRP) and fibrinogen, both defined as acute-phase proteins, are associated with atherosclerosis, metabolic disorders, and CV disease.[3]

Visceral obesity increases the concentration of plasminogen activator inhibitor type-1 (PAI-1), which is an inhibitor of t-PA (activator of plasminogen to plasmin).[4] Its inhibition results in a relative imbalance of fibrinolytic factors, which is associated with greater risk of thrombosis and higher risk of CV events.[5] Higher levels of fibrinogen, a zymogen that is activated to fibrin in blood clotting, are associated with MS[6] and T2DM,[7] but also with subclinical CV disease.[8]

CRP, a predictor of early atherosclerotic damage,[9] is related to high adiposity (mainly abdominal), IR and dyslipidemia. It directly promotes endothelial activation and impairs nitric oxide (NO) production, resulting in endothelial dysfunction.[10] Elevated CRP is also a marker of low-grade inflammation, especially when associated with visceral adipose tissue[11] and macrophage infiltration of the adipose tissue.[10,11] Adipocytokines may induce the production of CRP by the liver.

The biguanide metformin is an orally administered drug that lowers blood glucose in patients with T2DM by improving insulin sensitivity and suppressing hepatic gluconeogenesis.[12] It also reduces CV morbidity and mortality in overweight T2DM subjects[13] and, additionally, can lower the incidence rate of T2DM and MS in subjects with IGT.[14,15] However, even in normoglycemic subjects, there are also some clinical and experimental data that support its pleiotropic effects, mainly those related to

vascular tissue.[16] We have previously demonstrated its beneficial action in MS normoglycemic patients on both endothelial[17] and skin nutritive microvascular reactivity.[18] What still deserves investigation is whether markers of endothelial imbalance like CRP and fibrinogen may be used as markers of the beneficial effects of metformin in metabolic disorders. Our aim is to investigate if metformin acts on some classical markers of the CV risk profile, like the lipid profile, blood pressure, and fasting plasma glucose (FPG), and whether fibrinogen and CRP may be used as indirect markers of its action in first-degree relatives of T2DM subjects who have MS and normal glucose tolerance.

MATERIALS AND METHODS

All subjects were selected from the outpatient care facility of the State University of Rio de Janeiro. Thirty-one first-degree relatives of T2DM subjects who exhibit MS and normal glucose tolerance were recruited to the study, and they were randomly divided into placebo (n=15) and metformin (n=16) groups (Table 1). All subjects gave their written informed consent and our local Ethics Committee approved the protocol.

Anthropometric, clinical and laboratory measurements

The same trained examiner collected anthropometric measurements in duplicate at baseline and at the end of the treatment period. These included the waist size at its smallest point with the abdomen relaxed, and the body weight as measured using a digital scale (Filizola®, São Paulo, SP, Brazil). Body mass index (BMI) was defined as a patient's

Table 1 - Clinical, anthropometric and body composition characteristics of both groups at baseline and after treatment

	Placebo		Metformin	
	Baseline	After treatment	Baseline	After treatment
Gender (F/M)	9/6	-	13/3	-
Age (years)	37 [32-42]	-	40 [33.5-50]	-
Known hypertension	7[46.7%]	-	8[50%]	-
Anti-hypertensive drug use	5 [33%]	-	6[37.5%]	-
Weight (kg)	100.3 [84.6-109.9]	102.2† [86.2-111.6]	83.5 [78.6-96.7]	82.5* [77.5-96.4]
BMI (kg/m²)	36.7 [34.3-40.2]	37.2† [33.9-41.1]	34.2 [29.8-39.5]	33.6* [30.1-38.3]
Waist (cm)	104 [98-112]	105 [96-113]	97.5 [90.5-104.5]	97.5 [92.5-105.5]
Systolic BP	140 [126-149]	140 [124-145]	143 [123.5-149.5]	133.5* [124-141.5]
Diastolic BP	84.0 [75-91]	83.0 [71-88]	80.5 [73-87.5]	78.0 [72-87.5]
Fat Mass (%)	42.0 [37.0-46.0]	41.0 [35.0-45.0]	40.5 [38.0-43.0]	40.0 [35.5-43.0]
Fat Free Mass (%)	58.0 [54.0-63.0]	59.0 [55.0-65.0]	59.5 [57.0-62.0]	60.0 [57.0-64.5]

Data expressed as medians (1st-3rd quartiles). † $p < 0.05$ - comparisons within placebo group. *$p < 0.05$; ** $p < 0.01$ – comparisons within metformin group.

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weight in kilograms divided by squared height in meters. Body composition was analyzed using Vcorp software with QUANTUM II equipment (RJL systems Inc, USA), and required four measurements in duplicate. Mean values were used for analysis. Blood pressure (BP) was measured twice, with a five minute interval, at rest in the supine position, using an automated apparatus (Multiparameter patient monitor - Lifewindow LW6000, Digicare Biomedical Technology, West Palm Beach, FL, USA).

All laboratory measurements were performed in duplicate, after a 10-12 h fast, using an automated method (Modular Analytics PP, Roche, Basel, Switzerland). Mean values were used at analysis. Fasting and post-load plasma glucose, total cholesterol, triglycerides and high-density lipoprotein (HDL) cholesterol were measured, respectively, by enzyme-colorimetric GOD-PAP (inter-assay coefficient of variation (IECV) = 1.09%;), enzymatic GPO-PAP (IECV = 2.93%), enzymatic GPO-PAP (IECV = 1.29%) and enzyme-colorimetric method without pre-treatment (IECV = 3.23%). Plasma low-density lipoprotein (LDL) cholesterol was calculated according to the Friedwald equation. Fibrinogen (IECV = 4.33%) and CRP (IECV = 2.66%) were measured, respectively, using the coagulometric method and imunoturbidimetry with the modular Analytics P (ROCHE®) system. Insulin was monitored by electrochemiluminescence (IECV=10.6%,) and the homeostasis model assessment (HOMA-IR) was calculated to quantify insulin resistance.[19]

Study Design

During the first clinical visit, patients were subjected to a physical examination and were given a 75-g oral anhydrous glucose tolerance test (fasting and at 2 hours), a lipid profile assessment, and CRP and fibrinogen determination after a 10- to 12-hour fast. All subjects enrolled exhibited normal glucose tolerance test results according to the ADA criteria[20] and met at least three criteria for MS according to NCEP-ATPIII.[1]

The main exclusion criteria were pregnancy, T2DM or any degree of glucose intolerance, smoking, major illnesses, a history of previous myocardial infarction or angina pectoris, a post-menopausal history, and the use of oral contraceptives, aspirin, or statins.

Subjects were randomized 1:1 in a double-blinded fashion, in order to compare metformin to placebo. Pills were taken for at least 90 days. During the first week of treatment, only the dinner pill was taken, in order to minimize gastrointestinal side effects. After this period, pills were administered at lunch and at dinnertime. Metformin pills were prepared with 850 mg/pill by Merck-Santé, Lyon, France. Compliance was reconfirmed every 30 days. All subjects were asked to maintain their usual diet and physical activity. Antihypertensive drugs remained unchanged during the study.

Statistical Analysis

Comparisons between groups at baseline and after the treatment period were performed using the Mann-Whitney U test and the Wilcoxon matched pair tests, respectively. Frequency comparisons for the 2x2 tables were made with Yates corrected Chi-squared test. Differences noticed after the treatment period were transformed to percent of increment or decrement, and associations between these and the decrements in weight and BMI in the metformin group were assessed using a Spearman correlation. Sample size had not been calculated in advance and, as a consequence, the power of the reported analysis should be considered relevant only for the population studied. Significant differences were assumed to be present in cases when $p<0.05$. All group data are reported as the median [1st - 3rd quartiles].

RESULTS

Study population

There was no difference between the number of days of treatment for the placebo and metformin groups (109 [101-112] vs. 102.2 [97-112.5] days, p=0.21). Mean and maximum durations of treatment for the placebo and metformin groups were 106.7/116 and 102.5/116 days, respectively. Almost 50% of subjects in both groups had a known history of hypertension, and there was no difference between the groups in respect of this metric. At baseline, anthropometric, clinical, laboratorial and body composition measurements were no different between groups, except in the case of insulin and HOMA-IR (Table 2) data.

Anthropometric, clinical and laboratory data after treatment

There were no changes in either clinical or laboratory measurements in the placebo group, except for increasing weight and BMI (Table 1). The metformin group, however, had a decrease in weight, BMI, systolic BP, total and LDL-cholesterol and FPG (Tables 1 and 2) without changes in body composition (Table 1). The metformin group also exhibited increased HDL-cholesterol levels (Table 2). The two groups exhibited the same levels of CRP and fibrinogen at baseline (Table 2). After the treatment period, neither group had a CRP or fibrinogen level that was different from baseline (Table 2).

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Table 2 - Laboratory data for both groups at baseline and after treatment

	Placebo		Metformin	
	Baseline	After treatment	Baseline	After treatment
FPG (mg/dL)	87.8 [84.7-93.7]	90.9 [85.8-96.8]	93.7 [86.4-97.7]	89.2 [80.8-94.3]*
Post-load PG (mg/dL)	99.9 [88.9-108.9]	-	110.3 [99.9-122.7]	-
Total cholesterol (mg/dL)	186.42 [165.3-205.5]	199.6 [183.3-229.7]	209.4 [192.2-224.6]	197.3 [179.0-221.1]*
LDL-cholesterol (mg/dL)	120.9 [107.6-148.2]	134.9 [119.7-148.9]	131.0 [115.8-156.0]	120.5 [100.6-141.1]**
HDL-cholesterol (mg/dL)	36.2 [35.1-44.0]	39.00 [33.9-47.1]	40.1 [36.2-50.7]	47.1 [34.7-53.8]*
Triglycerides (mg/dL	122.8 [105.9-196.6]	118.37 [97.9-162.8]	162.8 [113.9-206.4]	186.01 [108.5-225.1]
Insulin (µUI/mL)	22.6 [13.0-26.4]	18.0 [9.6-23.8]	11.3 [10.9-17.0]†	12.3 [10.7-18.8]
HOMA-IR	4.08 [2.44-6.06]	3.09 [2.32-5.70]	2.49 [2.26-4.25]†	2.67 [2.13-4.43]
C-RP (mg/dL)	0.68 [0.3-1.2]	0.64 [0.3-1.0]	0.78 [0.3-1.1]	0.80 [0.4-0.9]
Fibrinogen (mg/dL)	282.2 [220.4-323.7]	286.7 [249.6-295.1]	303.9 [217.6-347.6]	290.9 [251.5-301.9]

Data expressed as medians (1st-3rd quartiles). †p<0.05 – comparison between placebo and metformin at baseline. *p < 0.05; ** p < 0.01 – comparisons within metformin group

Associations in the metformin group between decrements in weight and BMI and decrements in FPG, total and LDL-cholesterol and triglycerides, and increased HDL-cholesterol were tested, and there was no correlation between them.

DISCUSSION

Our findings demonstrate that short-term use of metformin in first-degree relatives of T2DM subjects who have MS and normal glucose tolerance promotes weight loss, an improved lipid profile, and better systolic BP and FPG (even within the normoglycemic range). CRP and fibrinogen are two acute-phase response proteins seen in inflammation,[3] and both are also associated with low-grade inflammation and atherogenesis. It was reasonable to suppose that metformin, a drug widely used in T2DM patients that exerts known protective effects on the vascular wall[16-18] and the inflammatory state,[21] would result in reduced levels of these two pivotal markers of inflammation and endothelial imbalance. However, this effect was not observed after the treatment period.

Metformin may directly impact the pathophysiology of athero-thrombosis, because in T2DM subjects its short-term use improved markers of endothelial dysfunction and inflammatory activity.[21] Since higher levels of adiposity are associated with low-grade inflammation, we expected that by losing weight and improving the CV risk profile, the use of metformin in MS subjects would change CRP and fibrinogen levels. This hypothesis could not be proved; therefore, these parameters should not be considered markers of CV disease risk response in our group.

The National Cholesterol Education Program's Adult Treatment Panel III report (NCEP-ATP III) classified MS as including six components related to CV disease: abdominal obesity, atherogenic dyslipidemia, raised BP, a pro-inflammatory state, a pro-thrombotic state, and IR[1]. Abdominal obesity, clinically presented as an increased waist circumference, is a major component of the MS and is linked to CV risk. This is due to an increase in several products, including nonesterified fatty acids (NEFA), cytokines, PAI-1, and lowered adiponectin levels.[22,23] The proinflammatory and prothrombotic states found in the MS are metabolically interconnected by high cytokine readings. The first is recognized clinically by an elevation of the CRP, which is related to obesity[2], mainly visceral. This relation shows that CRP screening is an important tool to identify increased risk in the case of CV morbidity and mortality. The prothrombotic state is characterized by increased PAI-1 and fibrinogen counts,[24] both of which are markers of an imbalance in the fibrinolytic system, with consequent injury to the vascular wall that contributes to vascular disease and CV risk.

The glucose-lowering effect of metformin is attributed mainly to decreased hepatic gluconeogenesis and enhanced peripheral glucose uptake.[12] The United Kingdom Prospective Diabetes Study showed that metformin use in overweight T2DM patients decreased macrovascular morbidity and mortality independent of glycemic control.[13] This suggests that metformin has pleiotropic vascular effects that act on endothelial imbalance, probably increasing NO bioavailability,[16] decreasing atheroma plaque growth, improving the atherogenic lipid profile,[25] inhibiting lipid incorporation into vessel walls, and inhibiting vascular smooth muscle cell proliferation.[26] Additionally, this drug acts directly on chronic heart disease by improving central hemodynamics parameters and functional class profiles,

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with a consequent improvement in quality of life.[27] This contributes new insight to the idea that metformin exerts not only vascular protective effects but also cardio-protective properties.[27-29]

Metformin improved the endothelium-dependent vasodilation not only in experimental[30] but also in T2DM patients.[31] Recently, it was suggested that the increased CV disease risk in individuals with IGT or T2DM was largely driven by the coexistence of multiple metabolic disorders rather than by hyperglycemia per se,[32] suggesting that the identification of clustering of metabolic abnormalities should be given more consideration in CV disease prevention. The use of antihypertensives and antihyperlipidemic drugs results in lower CV disease risk in T2DM subjects and also in non-diabetic patients, but questions remain regarding the use of insulin-sensitizers in subjects who are at high risk of CV disease, who have MS and IR, but who still exhibit a normal glucose tolerance state. Recently we reported that endothelial reactivity was improved by short-term use of metformin in persons with MS and normal glucose tolerance,[17] but long-term follow-up trials are lacking.

In disagreement with our negative results, in polycystic ovary syndrome and also in well-controlled T2DM with MS, it has been reported that a decrease in CRP follows metformin treatment,[33] and there was a greater decrease in CRP in treatment with metformin plus glibenclamide compared with the glibenclamide alone.[34] Consistent with our findings, others[21, 35] have demonstrated negative results regarding CRP levels. Recently, in non-obese T2DM patients, Lund and co-workers also failed to demonstrate any effects of metformin on fibrinogen as compared to repaglinide treatment.[36]

The cross-sectional design of the present study does not allow us to infer a causal relationship between the variables. Our data should be viewed as having a bi-directional cause-effect association that necessitates a new view of the markers of endothelial activation in the context of metabolic disorders and their responses to drugs. This also warrants further investigation. The limitations of our study are as follows. First, the short-term treatment durations may have influenced our endpoints. Although our data may point to possible long-term benefits, this should first be tested, before we could consider our results to have long-term implications. Both groups exhibited higher HOMA-IR and insulin levels that were unchanged after treatment, but the metformin group exhibited a higher insulin sensitivity as assessed by indirect measures at baseline compared to placebo. Whether this could have influenced our results is hypothetical and cannot be proven, although it may have biased our findings.

Although metformin improved FPG readings, even in the normoglycemic range, the fibrinogen level was in the normal range for both groups, and CRP levels were only slightly elevated and may even have been lower after the treatment period, paralleling our FPG results. It should thus be emphasized that our patients were selected according to criteria related to low-grade inflammation without any degree of IGT, and were possibly at precocious states of atherogenesis. Finally, both groups were kept on the same diet and underwent the same amount of physical activity during the study. Unfortunately, the placebo group gained weight, but ideally we would expect weight maintenance across both groups.

CONCLUSIONS

Metformin treatment improved some known risk factors for CV diseases, but did not alter levels of fibrinogen or CRP in first-degree relatives of T2DM subjects who have MS and normal glucose tolerance. Metformin demonstrated beneficial effects on the CV disease risk profile in short-term use. However, taking CRP and fibrinogen as markers of endothelium imbalance, we conclude on the basis of our findings that these two markers should not be used to monitor short-term treatment response to metformin.

ABBREVIATIONS: T2DM, type 2 diabetes mellitus; MS, metabolic syndrome; BMI, body mass index; IR, insulin resistance; CV, cardiovascular; FPG, fasting plasma glucose; BP, blood pressure; NO, nitric oxide; HOMA-IR, homeostasis assessment model for insulin resistance; IGT, impaired glucose tolerance; PAI-1, plasminogen activator inhibitor type-1.

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REFERENCES

1. Grundy SM, Brewer HB Jr, Cleeman JI, Smith SC Jr, Lenfant C; American Heart Association; et al. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Circulation. 2007;109:433-8.
2. Grundy SM. Obesity, Metabolic Syndrome, And Coronary Atherosclerosis. Circulation. 2002;105:2696-8.
3. Gabay C, Kushner I. Acute-Phase Proteins And Other Systemic Responses To Inflammation. N Engl J Med;1999;340:448-54.
4. Erickson LA, Fici GJ, Lund JE, Boyle TP, Polites HG, Marotti KR. Development Of Venous Occlusions In Mice Transgenic For The Plasminogen Activator Inhibitor-1 Gene. Nature;1990;346:74-6.
5. Hamsten A, De FU, Walldius G, Dahlen G, Szamosi A, Landou C, Et Al. Plasminogen Activator Inhibitor In Plasma: Risk Factor For Recurrent Myocardial Infarction. Lancet.1987;2:3-9.
6. Skalicky J, Muzakova V, Kandar R, Meloun M, Rousar T, Palicka V. Evaluation Of Oxidative Stress And Inflammation In Obese Adults With Metabolic Syndrome. Clin Chem Lab Med.2008;46:499-505.
7. Lund SS, Tarnow L, Frandsen M, Smidt UM, Pedersen O, Parving HH, Et Al. Impact Of Metformin Versus The Prandial Insulin Secretagogue, Repaglinide, On Fasting And Postprandial Glucose And Lipid Responses In Non-Obese Patients With Type 2 Diabetes. Eur J Endocrinol.2008;158:35-46.
8. Green D, Foiles N, Chan C, Schreiner PJ, Liu K. Elevated Fibrinogen Levels And Subsequent Subclinical Atherosclerosis: The CARDIA Study. Atherosclerosis. 2009;202:623-31.
9. Olsen MH, Christensen MK, Hansen TW, Gustafsson F, Rasmussen S, Wachtell K, Et Al. High-Sensitivity C-Reactive Protein Is Only Weakly Related To Cardiovascular Damage After Adjustment For Traditional Cardiovascular Risk Factors. J Hypertens. 2001;24:655-61.
10. Weisberg SP, Mccann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr., Et Al. Obesity Is Associated With Macrophage Accumulation In Adipose Tissue. J Clin Invest. 2003;112:1796-808.
11. Tall AR. C-Reactive Protein Reassessed. N Engl J Med. 2004;350:1450-2.
12. Bailey CJ. Biguanides And NIDDM. Diabetes Care.1992;15:755-72.
13. Effect Of Intensive Blood-Glucose Control With Metformin On Complications In Overweight Patients With Type 2 Diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. Lancet. 1998;352:854-65.
14. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Et Al. Reduction In The Incidence Of Type 2 Diabetes With Lifestyle Intervention Or Metformin. N Engl J Med. 2002;346:393-403.
15. Orchard TJ, Temprosa M, Goldberg R, Haffner S, Ratner R, Marcovina S, Et Al. The Effect Of Metformin And Intensive Lifestyle Intervention On The Metabolic Syndrome: The Diabetes Prevention Program Randomized Trial. Ann Intern Med. 2005;142:611-9.
16. Wiernsperger NF. Metformin: Intrinsic Vasculoprotective Properties. Diabetes Technol Ther. 2000;2:259-72.
17. De Aguiar LG, Bahia LR, Villela N, Laflor C, Sicuro F, Wiernsperger N, Et Al. Metformin Improves Endothelial Vascular Reactivity In First-Degree Relatives Of Type 2 Diabetic Patients With Metabolic Syndrome And Normal Glucose Tolerance. Diabetes Care. 2006;29:1083-9.
18. Kraemer De Aguiar LG, Laflor CM, Bahia L, Villela NR, Wiernsperger N, Bottino DA, Et Al. Metformin Improves Skin Capillary Reactivity In Normoglycaemic Subjects With The Metabolic Syndrome. Diabet Med. 2007;24:272-9.
19. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. C. Diabetologia;28:412-9.
20. American Diabetes Association. Diagnosis And Classification Of Diabetes Mellitus. Diabetes Care. 2009;32 Suppl 1:S62-7.
21. De JJ, Kooy A, Lehert P, Bets D, Wulffele MG, Teerlink T,Et Al. Effects Of Short-Term Treatment With Metformin On Markers Of Endothelial Function And Inflammatory Activity In Type 2 Diabetes Mellitus: A Randomized, Placebo-Controlled Trial. J Intern Med. 2005;257:100-9.
22. Cho YW, Yang DH, Oh DY, Baick SH, Kim SK, Kim SJ, Et Al. Plasma T-Pa And Pal-1 Antigen Concentrations In Non-Insulin Dependent Diabetic Patients: Effects Of Treatment Modality On Fibrinolysis. Korean J Intern Med. 1992;7:81-6.
23. Ozenoglu A, Balci H, Ugurlu S, Caglar E, Uzun H, Sarkis C, Et Al. The Relationships Of Leptin, Adiponectin Levels And Paraoxonase Activity With Metabolic And Cardiovascular Risk Factors In Females Treated With Psychiatric Drugs. Clinics. 2008;63:651-60.
24. Grant PJ. Beneficial Effects Of Metformin On Haemostasis And Vascular Function In Man. Diabetes Metab. 2003;29:6S44-52.
25. Ruan H, Lodish HF. Regulation Of Insulin Sensitivity By Adipose Tissue-Derived Hormones And Inflammatory Cytokines. Curr Opin Lipidol. 2004;15:297-302.
26. Hara T, Fujiwara H, Shoji T, Mimura T, Nakao H, Fujimoto S. Decreased Plasma Adiponectin Levels In Young Obese Males. J Atheroscler Thromb. 2003;10:234-8.
27. Lapina I, Narusov OI, Mareev VI, Bolotina MG, Shestakova MV, Masenko VP, Et Al. [Efficacy And Safety Of The Use Of Metformin In Patients With Chronic Heart Failure And Type 2 Diabetes Mellitus. Results Of The Study "Rational Effective Mulicomponent Therapy In The Battle Against Diabetes Mellitus In Patients With Chronic Heart Failure"]. Kardiologiia. 2008;48:58-68.
28. Masoudi FA, Inzucchi SE, Wang Y, Havranek EP, Foody JM, Krumholz HM. Thiazolidinediones, Metformin, And Outcomes In Older Patients With Diabetes And Heart Failure: An Observational Study. Circulation. 2005;111:583-90.
29. Eurich DT, Majumdar SR, Mcalister FA, Tsuyuki RT, Johnson JA. Improved Clinical Outcomes Associated With Metformin In Patients With Diabetes And Heart Failure. Diabetes Care. 2005;28:2345-51.
30. Katakam PV, Ujhelyi MR, Hoenig M, Miller AW. Metformin Improves Vascular Function In Insulin-Resistant Rats. Hypertension. 2000;35:108-12.
31. Mather KJ, Verma S, Anderson TJ. Improved Endothelial Function With Metformin In Type 2 Diabetes Mellitus. J Am Coll Cardiol. 2001;37:1344-50.
32. Liu J, Grundy SM, Wang W, Smith SC, Jr., Vega GL, Wu Z, Et Al. Ten-Year Risk Of Cardiovascular Incidence Related To Diabetes, Prediabetes, And The Metabolic Syndrome. Am Heart J. 2007;153:552-8.
33. Morin-Papunen L, Rautio K, Ruokonen A, Hedberg P, Puukka M, Tapanainen JS. Metformin Reduces Serum C-Reactive Protein Levels In Women With Polycystic Ovary Syndrome. J Clin Endocrinol Metab. 2003;88:4649-54.
34. Akbar DH. Effect Of Metformin And Sulfonylurea On C-Reactive Protein Level In Well-Controlled Type 2 Diabetics With Metabolic Syndrome. Endocrine. 2003;20:215-8.
35. Caballero AE, Delgado A, Guilar-Salinas CA, Herrera AN, Castillo JL, Cabrera T, Et Al. The Differential Effects Of Metformin On Markers Of Endothelial Activation And Inflammation In Subjects With Impaired Glucose Tolerance: A Placebo-Controlled, Randomized Clinical Trial. J Clin Endocrinol Metab. 2004;89:3943-8.
36. Lund SS, Tarnow L, Frandsen M, Smidt UM, Pedersen O, Parving HH, Et Al. Impact Of Metformin Versus The Prandial Insulin Secretagogue, Repaglinide, On Fasting And Postprandial Glucose And Lipid Responses In Non-Obese Patients With Type 2 Diabetes. Eur J Endocrinol. 2008;158:35-46.