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Treatment with insulin sensitizer metformin improves arterial properties, metabolic parameters, and liver function in patients with nonalcoholic fatty liver disease: a randomized, placebo-controlled trial

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ABSTRACT

Insulin resistance has an important role in the development of nonalcoholic fatty liver disease (NAFLD) and is involved in both pathological processes: hepatic steatosis and atherosclerosis. Therefore, treatment of NAFLD with insulin sensitizers is likely to have a favorable effect toward hepatic steatosis and cardiovascular outcomes. The present study investigated the effect of metformin on arterial properties, metabolic parameters, and liver function in patients with NAFLD. In a randomized, placebo-controlled study, 63 patients with NAFLD were assigned to 1 of 2 groups: Group 1 received daily metformin; group 2 received placebo. Pulse wave velocity (PWV) and augmentation index (AI) were measured using SphygmoCor (version 7.1; AtCor Medical, Sydney, Australia) at baseline and at the end of the 4-month treatment period. Metabolic measures and serum adiponectin levels were determined. Among metformin-treated patients, PWV and AI decreased significantly during the study. Significant declines in fasting glucose, triglyceride, and alkaline phosphatase and a significant increase in high-density lipoprotein cholesterol were observed. Alanine aminotransferase decreased and serum adiponectin increased marginally. In the placebo group, neither PWV nor AI improved significantly during the treatment period. Alanine aminotransferase, aspartate aminotransferase, and adiponectin did not change in the placebo group. Metformin treatment was associated with significant decrease in PWV and AI in NAFLD patients. This beneficial vascular effect was accompanied by an improvement in glucose and lipid metabolism as well as liver enzymes.

Trials Registry no.: NCT01084486.

Author contributions: Marina Shargorodsky contributed to the study conception and design. Elena Sofer, Marina Shargorodsky, and Margarita Mashavi were responsible for the data acquisition. Marina Shargorodsky and Mona Boaz performed the analysis and interpretation of data. Zipora Matas carried out the immunoassays. Marina Shargorodsky was responsible for reviewing the existing literature and for writing the first draft of the paper. All authors performed a critical revision of the manuscript for important intellectual content. All authors read and approved the final manuscript.

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1. Introduction

Nonalcoholic fatty liver disease (NAFLD), the most common cause of asymptomatic elevation of aminotransferase levels, is frequently associated with a range of cardiovascular risk factors including visceral obesity, dyslipidemia, insulin resistance, and type 2 diabetes mellitus [1-3]. Recent cross-sectional studies have shown that NAFLD is associated with increased carotid artery intima-media thickness [4,5] and decreased endothelium-dependent vasodilatation [6]. Moreover, the severity of histopathological features in NAFLD is strongly associated with carotid atherosclerosis [7]. Previous prospective studies reported that NAFLD is associated with an increased risk for future cardiovascular disease, independently of classic risk factors, liver enzymes, and the presence of metabolic syndrome [8].

There is no accepted standard medication in the treatment of NAFLD. Because insulin resistance has an important role in the pathophysiology of NAFLD, drugs that improve insulin sensitivity are widely used. Several clinical studies have shown significant improvement in insulin resistance and liver function as well as positive histological changes in patients treated with metformin and thiazolidinediones [9,10]. However, the long-term vascular impact of treatment, which improves insulin sensitivity in patients with NAFLD, has not been investigated. Estimation of arterial stiffness has been reported to be a useful method for assessment of early preclinical atherosclerosis. Pulse wave velocity (PWV) and augmentation index (AI), which are considered to be reliable and valid measures of vascular stiffness, are significantly and independently associated with target organ damage as well as cardiovascular morbidity and mortality. Measurement of arterial stiffness may serve as an indicator of treatment benefit [11-14].

Metformin is a potent insulin sensitizer and, as such, may alleviate the vascular damage caused by insulin resistance [15-17]. The present study was designed to evaluate the effect of metformin treatment on arterial properties, metabolic parameters, and liver function in patients with NAFLD.

2. Methods

2.1. Subjects

In a single-center study, 63 patients (32 men and 31 women) diagnosed with NAFLD were recruited from the outpatient clinic and evaluated for the study. The diagnosis of NAFLD was based on the results of abdominal ultrasonography and exclusion of viral-, autoimmune-, or drug-induced liver disease as well as alcohol intake of more than 20 g/d. Fatty liver disease was diagnosed on the basis on 4 sonographic criteria: (1) a diffuse hyperechoic echotexture (bright liver), (2) increased echotexture compared with the kidneys, (3) vascular blurring, and (4) deep attenuation [18].

Cardiovascular risk factors were defined using the National Cholesterol Education Program risk factors categories [19]: diabetes (fasting plasma glucose level ≥126 mg/dL and/or pharmacological treatment), hypertension (systolic blood pressure [SBP] ≥140 mm Hg and/or diastolic blood pressure [DBP] ≥90 mm Hg and/or pharmacological treatment), hypertriglyceridemia (≥150 mg/dL and/or pharmacological treatment), and low high-density lipoprotein (HDL) cholesterol level (<40 mg/dL in men and <50 mg/dL in women). *Impaired fasting glucose* was defined as fasting blood glucose greater than 100 mg/dL [20]. Screening procedures included physical examination, complete blood chemistry; complete blood count, urinalysis, and electrocardiogram. Patients with history of unstable angina, myocardial infarction, cerebrovascular accident, or major surgery within the 6 months preceding entrance to the study were excluded. Patients with unbalanced endocrine disease or any disease that might affect absorption of medications were excluded, as were patients with plasma creatinine greater than 1.5 mg/dL and electrolyte abnormalities. Patients included in the study were stabilized on their previous medical treatment in the outpatient clinic for up to 3 months, with an effort not to change treatment during the study to prevent possible effects on the study parameters.

Patients were randomly assigned to 1 of 2 groups: Group 1 received oral daily metformin at a dose of 850 to 1700 mg/d; group 2 received matching placebo capsules. Of the 63 patients recruited to the study, 52 completed the 4-month treatment period (27 from group 1 and 25 from group 2). This was a randomized, placebo-controlled, double-blinded study. Metformin therapy was generally well tolerated; only one patient from the metformin group withdrew because of gastrointestinal adverse effects. The reason for the rest of dropouts was loss to follow-up.

2.2. Informed consent

The study was approved by the regional ethical committee, and all procedures were performed in accordance with the guidelines of the Declaration of Helsinki. Informed written consent was obtained from all the subjects before participation. The study had been registered in ClinicalTrials.gov registry. The registration number is NCT01084486.

2.3. Biochemical parameters

Blood sampling for full chemistry and metabolic parameters, including fasting glucose, fasting insulin, lipid profile, high-sensitivity C-reactive protein (hs-CRP), liver function tests, and plasma adiponectin, was performed at baseline and at the end of the study. Adiponectin was determined by a commercial sandwich enzyme immunoassay technique (R&D Systems, Minneapolis, MN; catalog no. DRP300) with 2.8% intraassay and 6.5% interassay variability.

Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated by the following formula: fasting plasma insulin (in microunits per milliliter) × fasting plasma glucose (in milligrams per deciliter)/405. The HOMA β-cell function was calculated by using the following formula: 20 × fasting insulin (in microunits per milliliter)/fasting glucose (in millimoles per liter) − 3.5 [21,22]. Quantitative insulin sensitivity check index was calculated by the following formula: $1/[\log(I_0) + \log(G_0)]$.

2.4. BP and PWV measurement

Blood pressure was measured using an automated digital oscillometric device (Omron model HEM 705-CP; Omron, Tokyo, Japan), and a mean of 3 readings was taken. The radial pressure waveform was recorded and subsequently transformed by using a validated generalized transfer function incorporated in the SphygmoCor (version 7.1; AtCor Medical, Sydney, Australia) to give an estimate of the corresponding central ascending aortic pulse wave. With the integral software, the central augmented pressure (AP) was calculated as the difference between the early and late systolic peaks of the estimated central pressure waveform. Central aortic AI was calculated as the AP expressed as a percentage of the pulse pressure. Pulse wave velocity was measured by recording of the right carotid and the right radial artery pulse waveforms by 2 pressure transducers using the SphygmoCor Vx PWV System. This technique, which has been validated for its reproducibility and used extensively, estimates PWV between the 2 artery sites and had been accepted as substantially equivalent to aortic pressure measured by invasive catheterization [23]. Arterial stiffness was determined at baseline visit and at the end of the 4-month treatment period.

2.5. Statistical analysis

The sample size calculation was prospective. A sample size of 30 subjects in each group provided the present study with 91.5% power to detect a true, between-treatment difference of 0.70 ± 0.8 in end-point PWV assuming a 2-sided α of .05. This sample size also provided 81.5% power to detect a true between-treatment difference of 6 ± 8 in end-point AI assuming a 2-sided α of .05.

Analysis of data was carried out using SPSS 10.0 statistical analysis software (SPSS, Chicago, IL). For continuous variables, such as hemodynamic, biochemistry, and arterial elasticity parameters, descriptive statistics were calculated and reported as mean ± standard deviation. Normalcy of distribution of continuous variables was assessed using the Kolmogorov-Smirnov test (cutoff at $P = .01$). Categorical variables such as sex and comorbidities were described using frequency distributions and are presented as frequency (percentage).An intention-to-treat analysis and an on-treatment analysis ("per

Table 1 – Demographic and clinical characteristics of study patients

Variables	Metformin-treated subjects (n = 32)	Placebo-treated subjects (n = 31)
Male/female	17/15	14/17
Age (y)	51.9 ± 10.9	55.2 ± 14.0
BMI (kg/m²)	32.6 ± 5.8	31.5 ± 5.6
Current smokers, n (%)	4 (12.5%)	2 (6.5%)
Hypertension, n (%)	15 (46.9%)	15 (48.4%)
Dyslipidemia, n (%)	21 (65.6%)	14 (45.2%)
DM/IFG, n (%)	6/14(18.8%/43.8%)	2/13 (6.5%/41.9%)
Concomitant medication:		
Antidiabetic treatment (%)	6 (18.8%)	2 (6.5%)
Statins (%)	18 (56.3%)	11 (35.5%)
ACEIs/ARBs (%)	9 (28.1%)	9 (29.0%)
Diuretics (%)	1 (3.1%)	6 (19.4%)
β-Blockers (%)	9 (28.1%)	10 (32.3%)
CCB (%)	5 (15.6%)	8 (25.8%)
Aspirin (%)	13 (40.6%)	9 (29.0%)
Baseline SBP (mm/Hg)	138.8 ± 17.6	133.6 ± 18.1
Baseline DBP (mm/Hg)	80.1 ± 9.8	74.6 ± 13.5
Baseline fasting glucose (mg/dL)	131.8 ± 51.3	98.3 ± 14.8[†]
Baseline total cholesterol (mg/dL)	184.5 ± 43.3	191.1 ± 37.0
Baseline LDL cholesterol (mg/dL)	110.4 ± 37.9	112.7 ± 34.2
Baseline HDL cholesterol (mg/dL)	42.6 ± 13.0	48.2 ± 15.2
Baseline triglycerides (mg/dL)	185.5 ± 112.0	143.1 ± 63.2
Baseline hs-CRP (mg/dL)	0.9 ± 1.1	0.9 ± 1.4
Baseline AST (U/L)	28.9 ± 16.8	28.0 ± 8.2
Baseline ALT (U/L)	38.0 ± 29.9	32.6 ± 15.3
Baseline ALP (U/L)	67.7 ± 17.0	72.2 ± 23.5
Baseline creatinine (mg/dL)	0.9 ± 0.1	0.9 ± 0.2
Baseline urea (mg/dL)	32.0 ± 10.0	32.0 ± 9.0
Baseline adiponectin (μg/mL)	6.131 ± 2.873	9.156 ± 6.365[*]
Baseline PWV (m/s)	6.7 ± 1.1	6.3 ± 1.0
Baseline AI (%)	31.4 ± 11.1	30.0 ± 10.7

DM indicates diabetes mellitus; IFG, impaired fasting glucose; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; CCB, calcium channel blocker; LDL, low-density lipoprotein; ALP, alkaline phosphatase.

[‡] $P < .001$.

[*] $P < .05$.

[†] $P < .01$.

protocol") were performed. Because both of them were similar, the intention-to-treat analysis is presented. In addition, univariate general linear modeling (GLM) was used to compare outcomes by treatment assignment controlling for baseline values of covariates. Within a given treatment group, the *t* test for paired samples was used to compare before vs posttreatment values of outcomes. Categorical variables were compared between groups using the χ^2 test. All tests are 2-sided and considered significant at $P < .05$.

3. Results

Clinical and demographic characteristics of the study groups are presented in Table 1. As can be seen, the 2 groups were similar with respect to age, sex, presence of cardiovascular risk factors, baseline SBP and DBP levels, liver function, and arterial stiffness parameters. The 2 variables, fasting glucose and serum adiponectin levels, differed significantly by groups at baseline. Concomitant medications, which might affect arterial compliance parameters, like inhibitors of renin-angiotensin system and statins were equally distributed in both groups at the start and end of the study. None of the study participants had previously received metformin therapy.

3.1. *Changes in hemodynamic, arterial stiffness, and metabolic parameters in the metformin-treated patients*

Table 2 shows hemodynamic and arterial stiffness parameters of patients treated with metformin for 4 months. Systolic blood pressure and DBP did not change significantly during the study. Pulse wave velocity decreased significantly during the treatment period from 6.7 ± 1.1 to 5.8 ± 0.8 m/s ($P < .0001$). Augmentation index decreased from 31.4% ± 10.2% to 23.1% ± 8.5% ($P < .0001$), and aortic AP decreased from 15.6 ± 9.1 to 11.4 ± 7.6 mm Hg ($P = .001$).

As shown in Table 2, significant declines in fasting glucose, triglycerides, and alkaline phosphatase together with a significant increase in HDL cholesterol were observed in metformin-treated patients. C-reactive protein and alanine aminotransferase (ALT) decreased marginally during the 4-month treatment period. Serum adiponectin level tended to increase during the treatment period with metformin; however, this increase did not reach statistical significance.

3.2. *Changes in hemodynamic, arterial stiffness, and metabolic parameters in the placebo group*

As shown in Table 2, neither PWV nor AI changed significantly during the study. No change was detected in either SBP or DBP, whereas aortic AP increased marginally during the follow-up period. Alanine aminotransferase, aspartate aminotransferase (AST), and serum adiponectin levels did not change in placebo group.

3.3. *Between-group comparisons*

As can be seen in Table 1, both patient groups were similar at baseline in terms of arterial stiffness parameters. Systolic BP and DBP were similar in both groups at baseline as well at the end of the study. Although PWV and AI did not differ significantly between the groups at baseline, at the end of the study, PWV and AI were significantly lower in patients treated with metformin than in the placebo group ($P = .003$ and $P = .038$, respectively) (Fig. 1A, B). Because fasting glucose and serum adiponectin levels differed significantly by groups at baseline, univariate GLM analysis was carried out to control for these findings. Baseline mean arterial pressure (MAP), as a

Table 2 – Change from baseline in hemodynamic, arterial stiffness, and metabolic variables in each group

Variable	Metformin-treated subjects			Placebo-treated subjects		
	Baseline	4 mo	*P* value	Baseline	4 mo	*P* value
SBP (mm/Hg)	139.0 ± 17.9	137.2 ± 20.1	.565	133.0 ± 19.5	139.8 ± 19.3	.091
DBP (mm/Hg)	80.6 ± 9.2	78.4 ± 9.0	.403	73.3 ± 14.1	75.0 ± 9.4	.589
Aortic AP (mm/Hg)	15.6 ± 9.1	11.4 ± 7.6	.001	15.6 ± 9.3	20.2 ± 18.6	.271
AI (%)	31.4 ± 10.2	23.1 ± 8.5	<.0001	29.9 ± 11.0	29.0 ± 11.5	.625
PWV (m/s)	6.7 ± 1.1	5.8 ± 0.8	<.0001	6.3 ± 1.0	6.5 ± 1.0	.441
Creatinine (mg/dL)	0.9 ± 0.1	0.9 ± 0.2	.481	0.9 ± 0.2	0.9 ± 0.2	.627
Urea (mg/dL)	32.3 ± 10.5	31.3 ± 9.0	.569	31.2 ± 7.6	31.1 ± 10.5	.960
Total cholesterol (mg/dL)	179.3 ± 40.3	176.7 ± 32.2	.722	190.0 ± 38.5	186.2 ± 32.6	.616
Triglycerides (mg/dL)	195.4 ± 119.1	157.9 ± 97.4	.033	140.8 ± 58.0	135.0 ± 57.6	.588
HDL cholesterol (mg/dL)	41.3 ± 12.2	45.7 ± 15.0	.001	47.8 ± 14.3	49.6 ± 15.3	.329
LDL cholesterol (mg/dL)	103.5 ± 37.3	103.7 ± 22.1	.979	110.8 ± 35.2	109.0 ± 25.7	.786
ALP (U/L)	66.5 ± 17.8	61.1 ± 15.6	.007	74.3 ± 24.6	67.4 ± 19.6	.065
ALT (U/L)	38.5 ± 31.7	29.3 ± 16.2	.092	34.9 ± 15.8	29.7 ± 16.3	.120
AST (U/L)	29.0 ± 18.0	25.4 ± 9.7	.300	29.4 ± 8.4	27.4 ± 8.3	.246
hs-CRP (mg/dL)	1.0 ± 1.1	0.5 ± 0.4	.081	1.0 ± 1.5	0.5 ± 0.5	.139
Fasting glucose (mg/dL)	135.0 ± 52.8	115.8 ± 27.0	.036	98.1 ± 15.9	103.9 ± 18.5	.015
Adiponectin (μg/mL)	5.662 ± 2.820	6.167 ± 3.265	.171	10.252 ± 6.610	10.020 ± 5.648	.699
HOMA-IR	7.2 ± 6.3	7.3 ± 10.9	.920	5.3 ± 5.1	6.1 ± 8.0	.525
HOMA-β	202.05 ± 131.4	177.3 ± 87.0	.338	216.7 ± 168.7	178 ± 138.1	.096
QUICKI	0.302 ± 0.026	0.304 ± 0.025	.706	0.321 ± 0.037	0.317 ± 0.035	.330

QUICKI indicates quantitative insulin sensitivity check index.

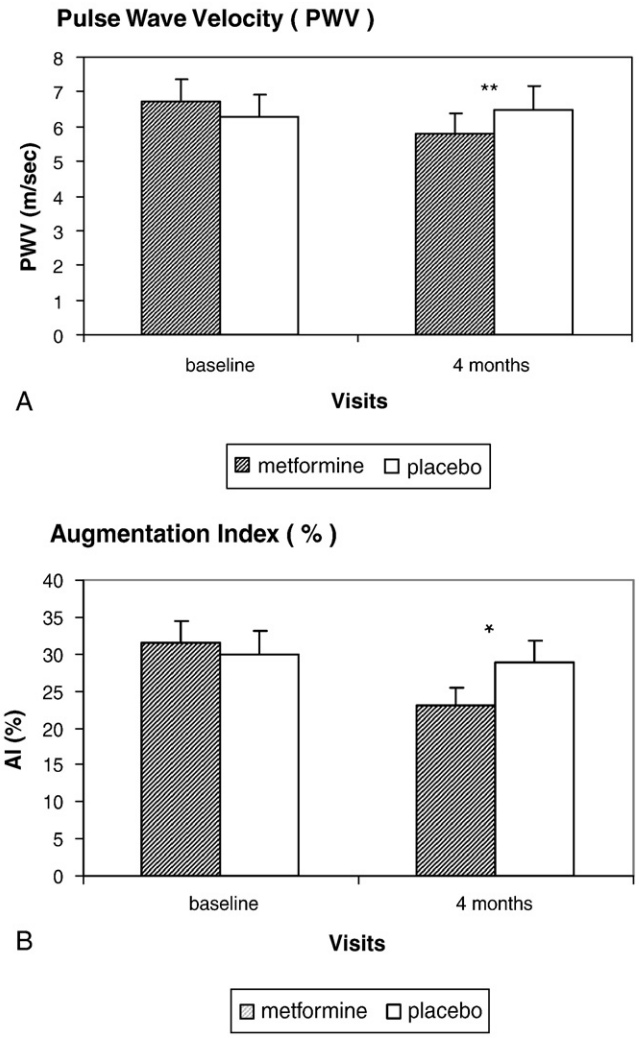

Fig. 1 – A, Pulse wave velocity by group during the 4-month follow-up. *P < .05, **P < .01, and ***P < .001. Values are means ± SD. B, Augmentation index by group during the 4-month follow-up. *P < .05, **P < .01, and ***P < .001. Values are means ± SD.

representative of hemodynamic values, was also included as a covariate in this model. Significant by-group differences in PWV persisted even after adjustment for baseline values of MAP, fasting glucose, and serum adiponectin levels ($P = .001$). The model is significant ($P < .0001$) and explains almost 37% of the variability in end-point PWV. The above-mentioned variables were also entered into a univariate GLM model of AI; and significant by-group differences in AI persisted after controlling for baseline values of MAP, fasting glucose, and serum adiponectin levels ($P = .037$). The total model was significant ($P < .0001$) and explained more than 49% of the variability in AI at end point.

3.4. *General linear model of end-point PWV and AI*

To determine whether the end-point measures of arterial stiffness result from improved glycemic control in the active treatment group or from exposure to metformin per se, change from baseline glucose was included in the general linear model of end-point PWV and, separately, AI. Included in each of these models were age, sex, baseline MAP, baseline adiponectin, and baseline PWV or AI (depending on the model). In addition, metformin exposure was included as a fixed factor in both models. The model of end-point PWV was significant and explained 35.5% of the variability in this outcome. Significantly associated with end-point PWV were baseline PWV ($P = .005$) and exposure to metformin ($P = .002$). Change from baseline glucose was not associated with this outcome ($P = .292$). The model of end-point AI was significant and explained approximately 64.5% of the variability in this outcome. Change from baseline glucose was not associated with this outcome ($P = .344$).

To further isolate the effect of changes in glycemic control on measures of arterial stiffness, results were examined in subjects without diabetes. In this subgroup, although baseline arterial stiffness parameters were similar in both groups, end-point PWV was significantly lower in patients treated with metformin than in the placebo group (5.8 ± 0.8 vs 6.5 ± 0.9, $P = .003$). End-point AI was significantly lower in metformin-treated subjects compared with the placebo group (23.1 ± 8.5 vs 29.0 ± 11.5, $P = .038$).

4. Discussion

The present randomized, placebo-controlled study demonstrates that metformin treatment was associated with a significant decrease of PWV and AI in patients with NAFLD. This beneficial vascular effect was associated with an improvement in glucose and lipid metabolism and liver function. The metformin treatment was safe in this study population in addition to being effective. To the best of our knowledge, the present study is the first to estimate an effect of treatment with the insulin sensitizer metformin on arterial stiffness in subjects with NAFLD.

Insulin resistance is involved in both hepatic steatosis and atherosclerosis; so it is not surprising that NAFLD is associated with increased carotid artery intima-media thickness and increased risk of future cardiovascular disease [7,8]. This also forms the logic underpinning treating NAFLD with a drug that increases insulin action. Previous prospective studies have reported improvement in biochemical and histological features of NAFLD with insulin sensitizers such as pioglitazone and rosiglitazone [24,25].In addition, improved insulin sensitivity and decreased liver volume have been observed during long-term metformin therapy [26]. The present study has shown for the first time that metformin treatment not only improved metabolic parameters and liver function, but also significantly decreased arterial stiffness, a valid marker of generalized atherosclerosis.

Although the antiatherogenic effect of metformin has been assessed previously, the mechanisms by which this agent inhibits atherosclerosis and hepatic steatosis remain to be clarified. Metformin decreases hepatic glucose output and enhances peripheral glucose uptake [27]. Metformin treatment increases insulin action in peripheral tissue, resulting in improvements in endothelium-dependent vasodilatation and decreases in the local activity of growth factors in vascular tissue, which in turn decrease the development of vascular

smooth muscle cell hypertrophy [15,28,29]. It has been also shown that metformin reduces elevated levels of plasminogen activator inhibitor 1 and factor VII; augments fibrinolysis; stabilizes platelets [17]; and has a beneficial effect on nitroxidation, endothelial function, and intima-media thickness in patients with metabolic syndrome [30]. In addition, metformin activates adenosine monophosphate–activated protein kinase in hepatocytes as well as phosphorylation of acetyl–coenzyme A carboxylase, resulting in decreasing hepatic lipogenesis [31].

Because our study detected significant declines in fasting glucose in metformin-treated patients, it has been questioned whether end-point measures of arterial stiffness result from improved glycemic control or from exposure to metformin per se. In univariate GLM analysis, changes in fasting glucose from baseline value were not associated with end-point PWV as well as end-point AI. Furthermore, in additional analysis that included nondiabetic subjects only, end-point PWV and AI were significantly lower in patients treated with metformin than in the placebo group. These data emphasize a direct beneficial effect of metformin on vascular function in patients with NAFLD, although the precise mechanisms for metformin action on the vasculature remain to be elucidated.

In the present study, we did not observe significant changes in adiponectin, which has recently been considered as a possible link between liver dysfunction and atherosclerotic vascular disease in patients with NAFLD [32]. Nevertheless, serum adiponectin level tended to increase in metformin-treated patients during the 4-month treatment period. Previously, significant improvement in serum adiponectin levels after 12 months of treatment with the insulin sensitizer pioglitazone in subjects with NAFLD has been reported [33]. Therefore, it seems reasonable to suppose that the increase in adiponectin levels requires more time under conditions of improved glucose tolerance and amelioration of hyperlipidemia and a proinflammatory state.

Moreover, it has been demonstrated that pioglitazone treatment increased plasma adiponectin level, whereas metformin did not significantly affect plasma adiponectin, in patients with type 2 diabetes mellitus [34].

4.1. *Limitations*

The present study has some limitations. Our study includes a relatively small number of participants, and larger studies are required to establish the beneficial vascular effect of metformin as well as its clinical impact on cardiovascular outcomes in subjects with NAFLD. In addition, the diagnosis of NAFLD was not confirmed by liver biopsy for ethical reasons. Although the criterion standard for the diagnosis of NAFLD remains liver histology, it has been shown that, after exclusion of the other common liver diseases, ultrasound examination is a reliable noninvasive method for the diagnosis of fatty liver [18].

4.2. *Conclusion*

We have demonstrated that metformin treatment improves arterial stiffness parameters in patients with NAFLD. The findings of the present study suggest that the beneficial vascular effect of metformin may lead to a decrease in future cardiovascular events in this population, but they will need to be confirmed by additional long-term follow-up studies.

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