Does Metformin Treatment Influence Bone Formation in Patients with Nonalcoholic Fatty Liver Disease?

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Abstract

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Antidiabetic drug metformin that improves insulin sensitivity and used in the treatment of nonalcoholic fatty liver disease (NAFLD), may affect the bone health. Our study was designed to investigate a possible effect of metformin on bone formation marker, procollagen type I N-terminal propeptide (P1NP) in patients with NAFLD. In a randomized, placebo controlled study, 63 patients with NAFLD were assigned to one of 2 groups: Group 1 received daily metformin and Group 2 received placebo. Metabolic parameters, insulin resistance markers, and P1NP were determined. Although circulating P1NP levels did not differ significantly between the groups at baseline, at the end of the study, P1NP was significantly lower in patients treated with metformin than in the placebo group ($p < 0.007$). Within-group analysis indicated that P1NP levels significantly decreased ($p = 0.023$) in patients receiving metformin during 4-month follow-up period, while no change in P1NP was observed in placebo group ($p = 0.359$). In general linear model metformin treatment was the only significant independent predictor of endpoint P1NP. Metformin treatment was associated with decrease in P1NP levels in patients with NAFLD. The effect on P1NP was independent of glucose lowering effect and caused from exposure to metformin per se.

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Introduction

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Accumulating evidence indicates that nonalcoholic fatty liver disease (NAFLD) is associated with low bone mass in both children and adult populations and may act as a risk factor for a osteoporosis-related bone fractures [1,2]. The pathogenesis of osteoporosis in patients with nonalcoholic fatty liver disease is multifactorial and still not completely understood. Recently it has been shown that circulating markers of bone metabolism, including osteoprotegerin, osteopontin, osteocalcin, and fetuin-A, have been altered in patients with NAFLD [3–5]. Moreover, antidiabetic drugs such as thiazolidinediones and metformin that improve insulin sensitivity and used in the treatment of NAFLD, may affect the bone health. Although most experimental data demonstrated that metformin has a direct osteogenic effect [6–8], the results of clinical trials are equivocal. Metformin treatment has been shown to reduce the association between diabetes and fractures in type 2 diabetic patients [9]. However, significant decrease in markers of bone formation in diabetic patients treated with metformin compared to rosiglitazone was found [10].

In recent years, bone turnover markers with better performance have been assessed. The use of N-terminal propeptide of type 1 collagen, as a specific bone formation marker as well as reference analyte for bone turnover markers in clinical studies, was recommended by the International Osteoporosis Foundation and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) [11]. Our study was designed to investigate a possible effect of metformin on bone formation marker, PINP, in a specific study population in which this issue was not studied yet, that is, in patients with nonalcoholic fatty liver disease.

Subjects and Methods

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As previously reported, 63 patients (32 males and 31 females) diagnosed with nonalcoholic fatty liver disease were recruited from the outpatient clinic at the Wolfson Medical Center to participate in the study [12]. 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/day.

Patients with unbalanced endocrine disease or any disease that might affect absorption of medications were excluded, as were patients with plasma creatinine > 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. None of the study participants had previously received metformin therapy. Patients were randomly assigned to one of the 2 groups: Group 1 received oral daily metformin at a dose of 850–1 700 mg/day and 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). The study was a randomized, placebo controlled. Metformin therapy was generally well tolerated; only one patient from metformin group withdrew because of gastrointestinal side effects. The reason for the rest of dropouts was loss to follow-up.

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: NCT01084486.

Biochemical parameters

Blood sampling for full chemistry and metabolic parameters, including fasting glucose, fasting insulin, lipid profile, CRP, fibrinogen, and liver function tests was performed at baseline and at the end of the study. The bone formation marker procollagen type I N-terminal propeptide (P1NP) were determined by electrochemiluminescence Immunoassaay (Roche). Adiponectin was determined by a commercial sandwich enzyme immunoassay technique [R&D Systems, Minneapolis, USA (Catalogue number DRP300)] with 2.8 % intra-assay and 6.5 % inter-assay variability.

Statistical analysis

Analysis of data was carried out using SPSS 10.0 statistical analysis software (SPSS Inc., Chicago, IL, USA). 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 $p = 0.01$). Categorical variables such as sex and co-morbidities were described using frequency distributions and are presented as frequency (%). Additionally, univariate general linear modeling (GLM) was used to compare outcomes by treatment assignment controlling for values of covariates Within a given treatment group, the t-test for paired samples was used to compare before vs. post-treatment values of outcomes. Categorical variables were compared between groups using the chi-square test. All tests are 2-sided and considered significant at $p < 0.05$.

Results
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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, gender, presence of cardiovascular risk

**Table 1** Demographic and clinical characteristics of study patients.

Variables	Metformin-treated patients (n = 32)	Placebo group (n = 31)	p-Value
Male/Female	17/15	14/17	0.352
Age (years)	51.9 ± 10.9	55.2 ± 14.0	0.306
BMI (kg/m²)	32.6 ± 5.8	31.5 ± 5.6	0.410
Current smokers, n (%)	4 (12.5 %)	2 (6.5 %)	0.391
Hypertension, n (%)	15 (46.9 %)	15 (48.4 %)	0.599
Dyslipidemia, n (%)	21 (65.6 %)	14 (45.2 %)	0.079
DM/IFG, n (%)	6/14 (18.8 %/43.8 %)	2/13 (6.5 %/41.9 %)	0.128
Concomitant medication			
Antidiabetic treatment (%)	6 (18.8 %)	2 (6.5 %)	0.194
Statins (%)	18 (56.3 %)	11 (35.5 %)	0.079
ACEIs/ARBs (%)	9 (28.1 %)	9 (29.0 %)	0.609
Diuretics (%)	1 (3.1 %)	6 (19.4 %)	0.104
β-blockers (%)	9 (28.1 %)	10 (32.3 %)	0.784
CCB-blockers (%)	5 (15.6 %)	8 (25.8 %)	0.351
Aspirin (%)	13 (40.6 %)	9 (29.0 %)	0.292
Baseline systolic BP (mm Hg)	138.8 ± 17.6	133.6 ± 18.1	0.258
Baseline diastolic BP (mm Hg)	80.1 ± 9.8	74.6 ± 13.5	0.072
Baseline fasting glucose (mg/dl)	131.8 ± 51.3	98.3 ± 14.8	0.034
Baseline total cholesterol (mg/dl)	184.5 ± 43.3	191.1 ± 37.0	0.523
Baseline LDL Cholesterol (mg/dl)	110.4 ± 37.9	112.7 ± 34.2	0.811
Baseline HDL-cholesterol (mg/dl)	42.6 ± 13.0	48.2 ± 15.2	0.123
Baseline triglycerides (mg/dl)	185.5 ± 112.0	143.1 ± 63.2	0.070
Baseline hs-CRP (mg/dl)	0.9 ± 1.1	0.9 ± 1.4	0.969
Baseline AST (U/l)	28.9 ± 16.8	28.0 ± 8.2	0.796
Baseline ALT (U/l)	38.0 ± 29.9	32.6 ± 15.3	0.377
Baseline ALP (U/l)	67.7 ± 17.0	72.2 ± 23.5	0.386
Baseline creatinine (mg/dl)	0.9 ± 0.1	0.9 ± 0.2	0.723
Baseline HOMA-IR	7.0 ± 6.9	5.4 ± 4.9	0.284
Baseline P1NP (µg/l)	39.8 ± 16.4	41.3 ± 12.1	0.754

factors, and liver function. As can be seen from ☉ **Table 1**, both groups were similar at baseline in terms of P1NP levels, hemodynamic, and metabolic parameters. Although circulating P1NP levels were did not differ significantly between the groups at baseline (p=0.754), at the end of the study, P1NP was significantly lower in patients treated with metformin than in the placebo group (p<0.007) (☉ **Fig. 1**).

As shown in ☉ **Table 2**, within-group analysis indicated that P1NP levels significantly decreased from 38.8±16.3 to 26.0±9.6 (p=0.023) in patients treated with metformin during 4-month follow-up period, while no change in P1NP was observed in placebo group (p=0.359). Adiponectin levels tended to increase in metformin-treated patients, however, this increase did not reach statistical significance (p<0.171). In placebo group adiponectin levels did not change during 4-month follow-up (p<0.699).

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) did not change significantly during the follow-up in both study groups.

Significant declines in fasting glucose, triglycerides, and alkaline phosphatase together with a significant increase in HDL cholesterol were observed in metformin-treated patients. CRP and ALT decreased marginally during 4-month treatment period. Among metabolic parameters, ALT, AST, and alkaline phosphatase levels did not change in placebo group during 4-month follow-up.
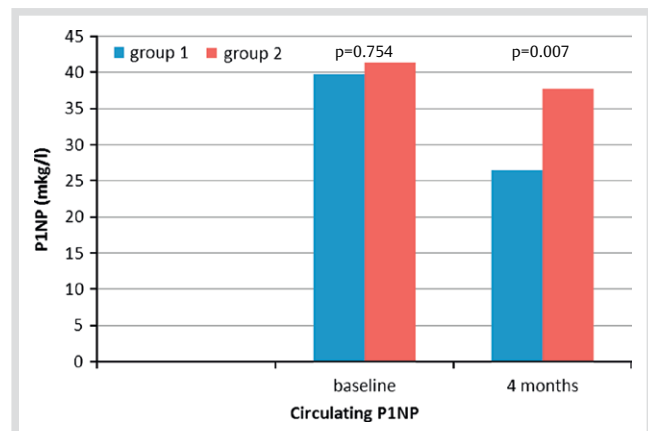
Fig. 1   Circulating P1NP by groups during 4-month follow-up. (Color figure available online only).

Discussion
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The present randomized, placebo-controlled study demonstrates that metformin treatment was associated with a significant decrease in P1NP levels in patients with nonalcoholic fatty liver disease. The effect on P1NP was independent of glucose lowering effect of metformin and caused from exposure to metformin per se.

To the best of our knowledge, the present study is the first to estimate a relation of insulin sensing effect of metformin to changes in bone remodeling as determined by P1NP level in patients with diagnosed NAFLD.

Findings of the present study concur with a published study, which showed significant reduction in serum markers of bone formation such as P1NP and bone alkaline phosphatase for both women and men with type 2 diabetes mellitus treated with rosiglitazone, metformin, and glyburide for 12 months, with the greatest changes in the metformin group (males: bone alkaline phosphatase, −16.4%, P1NP, −19.3%; females: bone alkaline phosphatase, −15.7%, P1NP, −14.4%) [13]. Moreover, it has been shown that in patients with poorly controlled type 2 diabetes, bone-specific alkaline phosphatase was decreased, whereas osteocalcin (OC) was increased after glycemic control for a month [14].

In the present study, the effect on P1NP was independent of glucose lowering effect of metformin. Since significant declines in fasting glucose were observed in metformin group, it has been questioned whether reduction in P1NP reflects the improvement in glucose homeostasis. To determine whether the endpoint measures of P1NP result from exposure to metformin per se, general linear model of endpoint P1NP was carried out. This analysis was modeled using multiple linear regression analysis with a backward, stepwise approach. For inclusion, the probability of f was set at 0.05 for entry and at 0.10 for exclusion. Included in the model of endpoint P1NP were age, sex, and post-treatment fasting blood glucose. Additionally, metformin exposure was included as a fixed factor. In this model, group was the only significant independent predictor of endpoint P1NP (p=0.031).

Recent experimental and clinical data have shown that metformin is osteogenic in vitro. It was reported that metformin can induce MC3T3-E1 osteoblastic cells differentiation and bone matrix synthesis via adenosine 5′-monophosphate-activated

Table 2   Change from baseline in homodynamic, arterial stiffness, and metabolic variables in each group.

Variables	Metformin treated patients			Placebo group		
	Baseline	4-month	p-value	Baseline	4-month	p-value
Systolic BP (mm Hg)	139.0±17.9	137.2±20.1	0.565	133.0±19.5	139.8±19.3	0.091
Diastolic BP (mm Hg)	80.6±9.2	78.4±9.0	0.403	73.3±14.1	75.0±9.4	0.589
Creatinine (mg/dl)	0.9±0.1	0.9±0.2	0.481	0.9±0.2	0.9±0.2	0.627
Urea (mg/dl)	32.3±10.5	31.3±9.0	0.569	31.2±7.6	31.1±10.5	0.960
Total Cholesterol (mg/dl)	179.3±40.3	176.7±32.2	0.722	190.0±38.5	186.2±32.6	0.616
Triglycerides (mg/dl)	195.4±119.1	157.9±97.4	0.033	140.8±58.0	135.0±57.6	0.588
HDL-cholesterol (mg/dl)	41.3±12.2	45.7±15.0	0.001	47.8±14.3	49.6±15.3	0.329
LDL Cholesterol (mg/dl)	103.5±37.3	103.7±22.1	0.979	110.8±35.2	109.0±25.7	0.786
ALP (U/l)	66.5±17.8	61.1±15.6	0.007	74.3±24.6	67.4±19.6	0.065
ALT (U/l)	38.5±31.7	29.3±16.2	0.092	34.9±15.8	29.7±16.3	0.120
AST (U/l)	29.0±18.0	25.4±9.7	0.300	29.4±8.4	27.4±8.3	0.246
CRP (mg/dl)	1.0±1.1	0.5±0.4	0.081	1.0±1.5	0.5±0.5	0.139
Fasting glucose (mg/dl)	135.0±52.8	115.8±27.0	0.036	98.1±15.9	103.9±18.5	0.015
HOMA-IR	7.2±6.3	7.3±10.9	0.920	5.3±5.1	6.1±8.0	0.525
Adiponectin (ng/ml)	5662±2820	6167±3265	0.171	10282±6610	10020±3265	0.699
P1NP (mcg/l)	38.8±16.3	26.0±9.6	0.023	41.3±12.1	37.8±13.9	0.359

protein kinase (AMPK) activation and subsequent induction of endothelial nitric oxide synthase (eNOS) and bone morphogenetic protein-2 (BMP-2) expression [6, 15]. Metformin was also found to increase osteoblast proliferation, alkaline phosphatase activity and the number of mineralized nodules formed in rat primary osteoblasts, possibly via stimulation of Runx2 and IGF-1 production [16]. In contrast, 2 in vitro studies have shown no effect of metformin on the osteogenic differentiation of bone marrow-derived mesenchymal stem cells (MSCs) and matrix mineralization of both MC3T3-E1 cells and primary osteoblasts. Moreover, high concentration of metformin even clearly inhibited osteoblast differentiation [17, 18].

Less work has investigated the effect of metformin on bone in vivo, and the data are equivocal. It was reported that 2 months of treatment with metformin prevents the bone loss induced by ovariectomy in rats, suggesting protective effects of metformin against bone loss [19, 20]. A very recent study performed in insulin-resistant mice also showed that metformin given for 6 weeks protects femoral bone architecture compared to rosiglitazone, although metformin had no effect on lumbar spine [21]. Nevertheless, it was shown that 1- to 2-month treatment with metformin, regardless of the administration route, has no significant effect on rodent bone architecture and fracture healing in vivo and that metformin significantly reduces bone formation rate in osteopenic trabecular bone [22]. Further, markers of bone formation were decreased in the metformin group compared to the rosiglitazone one in T2DM patients from the ADOPT study [13]. Given these discrepant findings, future studies should clarify the clinical impact of reduced P1NP during metformin treatment on bone density as well as fragility fractures in NAFLD patients.

Additionally, since the present study focused on P1NP assessment, the effect of metformin treatment on additional markers of osteoblastic differentiation such as osteocalcin as well as bone specific ALP will need to be evaluated by long-term studies in this population.

In conclusion, we have demonstrated that metformin treatment may affect bone formation in patients with nonalcoholic fatty liver disease. The findings of the present study justify further controlled, long-term studies with measurement of bone markers, bone density, and fractures rates to demonstrate overall clinical impact of decreased osteoblast-associated markers of bone formation in different populations, especially patients at increased risk for osteoporosis.

Conflicts of Interest
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The authors declare that they have no conflicts of interest in the authorship or publication of this contribution.

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