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Original article

Evaluation of metformin therapy using controlled attenuation parameter and transient elastography in patients with non-alcoholic fatty liver disease

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ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) is among the most common causes of liver disease worldwide. There is growing evidence on pathogenesis and pathophysiology of NAFLD. However, there is still no universally accepted pharmacotherapy protocol.
Methods: The study was conducted on 42 patients with NAFLD. They were randomized to dietary treatment alone (n = 21) or to diet and metformin therapy (n = 21). Liver ultrasonography, controlled attenuation parameter (CAP), liver stiffness (LS), complete blood count, anthropometric and biochemical parameters were obtained before treatment (baseline), and after 3 and 5 months of the therapy.
Results: Patients treated with diet and metformin exhibited significantly decreased CAP values at 3 and 5 months of the therapy compared to baseline (319 dB/m *vs.* 285 dB/m; $p < 0.05$; 319 dB/m *vs.* 295 dB/m; $p < 0.05$ respectively). Five months of diet and the metformin therapy resulted in significant reduction of LS value (6.2 kPa *vs.* 5.2 kPa; $p < 0.05$), while patients treated with diet alone had no significant changes in liver CAP and LS measurements.
Conclusions: Metformin therapy combined with dietary treatment seems to be effective for the reduction of hepatic steatosis and fibrosis. However, considering limitations of the study and inconsistent results of previous investigations in this area, there is a need for further research on metformin efficacy in this group of patients.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is among the most common causes of liver disease worldwide. Its estimated prevalence in general population ranges from 10% to 24%. However, as many as 57–74% of obese individuals develop NAFLD [1]. NAFLD encompasses a wide range of liver pathologies, from steatosis and nonalcoholic steatohepatitis (NASH) to cirrhosis. Over 15% of NAFLD cases progress to NASH [2]. Compelling evidence indicate that NAFLD is strongly associated with type 2 diabetes mellitus (T2DM) and abdominal obesity, it is also widely considered a hepatic manifestation of metabolic syndrome (MetS) [3,4].

High prevalence of NAFLD among general population urges a search for more specific and sensitive diagnostic tools. Serum aminotransferases have long been used as a surrogate marker of liver injury. However, NAFLD and NASH may progress without elevation of alanine aminotransferase (ALT) activity. Furthermore, ALT levels do not strongly correlate with liver disease severity [5]. Liver biopsy has been the gold standard for the assessment of liver fibrosis and steatosis for decades, but it is limited by its invasive nature, risk of complication, patient discomfort and sampling errors [6].

The most common technique employed for qualitative assessment of hepatic steatosis is ultrasonography (USG). Contrary to the liver biopsy, USG is noninvasive and widely available tool. It has been demonstrated that the sensitivity, specificity and positive predictive value of this technique to detect steatosis is as high as 80–100% [7]. However, while being reliable in qualitative assessment of hepatic steatosis, USG is not perfect in quantitative analysis. Controlled attenuation parameter (CAP) is a new ultrasound-based method for detection and quantification of

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hepatic steatosis, which has been found superior to classic USG. CAP also facilitates monitoring the efficacy of NAFLD therapies and disease progression [8].

To date, there have been no universally accepted guidelines for pharmacotherapy management in patients with NAFLD. Encouraging, though inconsistent results of metformin therapy have recently been reported. Due to its beneficial effects in NAFLD-associated comorbidities, such as impaired glucose levels, dyslipidemia and obesity, metformin might become generally accepted as a first-line treatment for NAFLD, as it is already for T2DM.

Metformin is a generally accepted first line-treatment in T2DM. It increases peripheral and hepatic insulin sensitivity, reduces basal liver glucose production and increases insulin-stimulated uptake and utilization of glucose by peripheral tissues [9]. In recent years new indications for metformin therapy have emerged, including polycystic ovary syndrome and obesity. Since NAFLD patients tend to suffer from impaired glucose levels, dyslipidemia and obesity, it has been suggested that metformin might have beneficial effect in hepatic steatosis. The precise mode of metformin's action in hepatic steatosis has not been entirely explored, but it is probably involved in disruption of mitochondrial oxidative process [10].

Patients and methods

It was an open-label, randomized, single-center study. All patients gave their written informed consent and the study was approved by the local ethics committee.

Patients and study protocol

Prior to the study initiation, 21 healthy volunteers (who did not fulfil the criteria of MetS and did not have features of steatosis in USG) were examined with TE in order to assess reference values of CAP. The study was conducted on 42 patients with MetS and hepatic steatosis. Candidates were considered eligible for the study if they were $\geq$ 18 years old, their CAP value exceeded 213 dB/m (the median CAP value in the reference group) and they met the criteria for MetS (defined as: waist circumference $\geq$ 94 cm for men and $\geq$ 80 cm for women; and 2 out of 4 following pathologies or medication to control them: increased blood pressure $\geq$ 130/85 mmHg, impaired glucose tolerance/diabetes, triglycerides serum concentration $\geq$ 150 mg/dl, reduced HDL-cholesterol $<$ 40 mg/dl in men or $<$ 50 mg/dl in women). In order to exclude other liver diseases, all patients underwent detailed clinical evaluation including laboratory tests: complete blood count, liver enzymes' activities (alanine aminotransferase [ALT], aspartate aminotransferase [AST]), gamma-glutamyltranspeptidase (GGTP), markers of viral hepatitides (hepatitis B surface antigen, anti-hepatitis C antibodies), lipid profile, bilirubin, creatinine, glucose and insulin serum level. A homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the formula: fasting insulin (mIU/ml) x fasting glucose (mg/dl) / 405.

The exclusion criteria were the following: previous use of metformin, insulin or other anti-diabetic medication, alcohol abuse defined as alcohol consumption exceeding 20 g/day for women and 30 g/day for men, other liver diseases (hepatitis B or C; autoimmune, genetic or metabolic liver disease), use of drugs known to induce liver steatosis (glucocorticoids, valproate, amiodarone, tamoxifen, calcium channel blockers, methotrexate), hypo- and hyperthyroidism, congestive heart failure, eGFR $<$ 60 ml/min/1.73m^2. Pregnancy, ascites and HIV infection were also considered as exclusion criteria.

Pharmacotherapy of concomitant diseases (hypertension, hyperlipidemia) initiated prior to the study was sustained.

Patients included in the study were randomized into two groups. Group 1 (n = 21) was put on a hypocaloric diet (1500 kcal), while Group 2 (n = 21) received metformin plus hypocaloric diet (1500 kcal). Metformin was started with a dose of 500 mg a day and it progressively increased at 2 weeks intervals to the maximum dose of 2000 mg a day. All participants were instructed in a low-calorie Mediterranean diet and performing medium-intensity aerobic exercise for 150 min/week.

Patients were evaluated for treatment intolerance and compliance with the recommendations (detailed interview, including analysis of "food and exercise notebook", pill count, weight measurement) after 3 and 6 weeks and then at 3 and 5 months of therapy. The treatment continued for 5 months, with clinical, laboratory and elastography follow-up after 3 and 5 months. All study participants completed 3 months of treatment. Afterwards 7 patients from the diet group dropped out due to noncompliance (n = 2), change of residence (n = 1), non-attendance at a follow up appointment (n = 4); 8 patients from the metformin group dropped out due to noncompliance (n = 4), pregnancy (n = 1), non-attendance at follow up appointment (n = 2) and starting glucocorticoid therapy (n = 1). Hence, an analysis after 5 months of treatment included 14 patients from Group 1 and 13 patients from Group 2.

The primary aim of the study was to evaluate absolute and relative changes in hepatic steatosis and fibrosis, using CAP and LS measurements. The secondary aim was to assess the effects of both therapies on anthropometric and laboratory parameters (weight, BMI; ALT, AST, GGTP, bilirubin, glucose, insulin, lipid profile, creatinine).

USG, LS and CAP measurements

Transient elastography (Fibroscan®) was used to measure CAP and LS for the assessment of hepatic steatosis and fibrosis, respectively. All patients were examined with 3.5 MHz standard M probe. LS and CAP were measured simultaneously and at the same volume of liver parenchyma at a depth between 25 mm and 65 mm. CAP was computed only if the associated LS was valid. The ultimate LS result was expressed in kPa and CAP in dB/m. In order to define a reference range of CAP values, TE was initially performed in 21 patients without MetS and no features of steatosis on USG. Based on the obtained results and in accordance with previous studies, the cut-off value for identifying hepatic steatosis and fibrosis was 213 dB/m [11] and $>$ 7.0 kPa [12], respectively.

All patients underwent traditional liver ultrasound by a single experienced operator. Hepatic steatosis was graded as mild (mild increase in liver echogenicity), moderate (increased liver echogenicity that obscured portal and hepatic vein walls) and severe (posterior attenuation of the deep liver parenchyma). TE and ultrasound were performed directly before treatment initiation, and then after 3 and 5 months of therapy.

Statistical analysis

All continuous variables were expressed as the mean values $\pm$ 95% confidence interval or median values $\pm$ interquartile range, as appropriate. Categorical variables were presented as numbers (proportions). Normality was verified using Shapiro-Wilk test. The groups were compared using Mann-Whitney U test. To examine the differences between quantitative variables Wilcoxon test was used. Correlation analyses were performed to examine the relationship between changes of CAP value and various clinico-laboratory parameters. A univariate and multivariable linear regression analysis was performed to evaluate the association between elastographic parameters and various clinico-laboratory factors (p values of less than 0.05 were considered significant. The data were analyzed using Statistica 12.0 (StatSoft Poland, Kraków).

Results

Baseline characteristics of the study population

At baseline, demonstrated clinical and demographic parameters were similar in both treatment groups. However, metformin-treated patients were younger (diet *vs.* metformin: 57 $\pm$ 13 years *vs.* 49 $\pm$ 11 years; $p < 0.05$) and more frequently on anti-hyperlipidemic therapy (diet *vs.* metformin: 42.9% *vs.* 76.2%; $p < 0.05$). Consequently, serum triglycerides levels differed between groups (diet - 104 [91-147] mg/dl *vs.* metformin - 152

Table 1
Baseline clinical data of patients.

Variable	All	Diet	Diet and metformin	*p*
Demographic data				
Sex – male/female, n (%)	22/20 (52.4/47.6)	13/8 (61.9/38.1)	9/12 (42.9/57.1)	0.2
Age, years, mean (95% CI)	53 (50–57)	57 (51–62)	49 (46–55)	0.02
Hypertension, n (%)	40 (95.2)	20 (95.2)	20 (95.2)	1
Type 2 diabetes mellitus, n (%)	12 (28.6)	5 (23.8)	7 (33.3)	0.4
IGT or IFG, n (%)	15 (35.7)	7 (33.3)	8 (38.1)	0.7
Hypercholesterolaemia, n (%) – *LDL≥115 mg/dl or pharmacotherapy*	28 (66.7)	13 (61.9)	15 (71.4)	0.6
Hypertriglyceridaemia, n (%) – *TG≥150 mg/dl or pharmacotherapy*	25 (59.5)	9 (42.9)	16 (76.2)	0.04
Anthropometric data				
Weight, kg, median (IQR)	85.5 (78.5–95.6)	82.9 (78.3–89.8)	88.2 (79.0–102.5)	0.2
BMI, kg/m^2, median (IQR)	30.6 (27.4–31.6)	29.2 (26.8–31.3)	31.4 (29.2–34.4)	0.07
Waist circumference, cm, median (IQR)	102.8 (98.0–109.5)	101.0 (96.0–106.0)	107.0 (102.0–118.0)	0.06
Hip circumference, cm, median (IQR)	108.0 (102.3–112.8)	104.0 (102.0–109.0)	112.0 (106.0–114.0)	0.02
WHR, mean (95% CI)	0.97 (0.95–0.98)	0.96 (0.94–0.99)	0.97 (0.95–0.99)	0.4

BMI, body mass index; IGT, impaired glucose tolerance; IFG, impaired fasting glucose; WHR, waist-to-hip ratio.
A p value of <0.05 is considered statistically significant.
p values were calculated with t-test or the Mann-Whitney U test, appropriately.

[103-226] mg/dl; $p < 0.05$). All patients were either overweight (50%) or obese (50%). Detailed clinical profile of the study population is presented in Tables 1 and 2.

The median baseline CAP of the whole study population was 316 dB/m [278-331] and it did not differ between the groups (diet *vs.* metformin: 309 dB/m [270-323] *vs.* 319 dB/m [302-342]. The median CAP of the reference group was significantly lower than in the group studied (213 dB/m [175-229]). LS was higher than 7.0 kPa in four patients of the diet group and seven patients in the metformin group.

Fourteen patients treated with diet only and eighteen patients on metformin therapy had ultrasonographic evidence of steatosis at baseline. CAP correlated with USG results in both study groups (r = 0.63, $p < 0.05$).

Primary outcome

Patients treated with metformin showed significantly decreased CAP after 3 and 5 months of therapy compared to the baseline (319 dB/m *vs.* 285 dB/m; $p < 0.05$; 319 dB/m *vs.* 295 dB/m; $p < 0.05$ respectively). After 3 months a mean CAP decrease of 14.3% was observed in 19 patients (90%) receiving metformin. After 5 months of metformin therapy all 13 patients who completed the study had lower fat liver content (mean decrease of 8.2%) compared to the baseline. Five months of metformin therapy also resulted in reduction of LS (6.2 kPa *vs.* 5.2 kPa; $p < 0.05$) – 8 out of 13 patients who completed the study had lower liver stiffness comparing to baseline (mean decrease of 26%).

Patients treated with diet alone had no significant changes in liver CAP and LS (Table 3).

After 3 months liver steatosis, as evaluated by USG, improved in 5 (24%) patients treated with diet alone and in 4 (19%) patients in the metformin group. Five months of metformin therapy resulted in further improvement of liver echogenity in 2 of these patients. There was no further improvement in the dietary treatment group.

Linear regression analysis

Change (Δ) in CAP after 3 months of treatment: The univariate linear regression analysis revealed that changes in CAP values were independently associated with metformin administration (β = 0.47; $p = 0.002$), weight change (β = 0.39; $p = 0.01$) and initial eGFR value (β = 0.32; $p = 0.04$). The multivariable linear regression analysis demonstrated that CAP change was associated only with metformin administration (β = 0.36; $p = 0.02$).

ΔCAP after 5 months of treatment: The univariate linear regression analysis revealed that CAP change was independently associated with metformin administration (β = 0.4; $p = 0.04$), hyperlipidaemia (β = -0.39; $p = 0.04$), weight change after 3 months of treatment (β = 0.44; $p = 0.02$) and baseline CAP value (β = -0.41; $p = 0.03$). The multivariable linear regression analysis demonstrated that CAP change was associated with weight change after 3 months of treatment (β = 0.57; $p < 0.001$) and hyperlipidemia (β = -0.3; $p = 0.03$).

Table 2
Baseline laboratory data of patients.

Variable	All	Diet	Metformin	*p*
Total cholesterol, mg/dl, mean (95% CI)	202.5 (189.6–215.4)	204.6 (188.66–220.5)	200.4 (178.7–222.2)	0.4
HDL cholesterol, mg/dl, median (IQR)	45 (37–56)	47 (41–62)	42 (36–47)	0.02
LDL cholesterol, mg/dl, median (IQR)	126 (100–154)	127 (107–145)	109 (95.75–159.75)	0.8
Triglycerides, mg/dl, median (IQR)	123.5 (94.25–167.75)	104 (91–147)	152 (103–226)	0.03
AST, U/l, median (IQR)	24.0 (20.25–30.5)	22 (20–29)	26 (21–34)	0.4
ALT, U/l, median (IQR)	30 (20–44)	24.0 (14.75–33.25)	31 (23–53)	0.2
GGTP, U/l, median (IQR)	36.5 (25.0–56.75)	35 (25–38)	61 (26–78)	0.07
Bilirubin, mg/dl, median (IQR)	0.43 (0.30–0.54)	0.42 (0.34–0.57)	0.43 (0.28–0.52)	0.9
Glucose, mg/dl, mean (95% CI)	105.4 (100.9–110.0)	102.7 (96.0–109.4)	108.3 (101.8–114.8)	0.4
Insulin, μU/ml, median (IQR)	10.5 (6.63–12.78)	8.2 (6.2–11.2)	11.2 (8.2–13.2)	0.08
HOMA – IR, median (IQR)	2.46 (1.80–3.20)	2.40 (1.72–2.53)	2.71 (2.04–3.29)	0.08
Creatinine, mg/dl, mean (95% CI)	0.81 (0.76–0.85)	0.84 (0.76–0.91)	0.78 (0.73–0.84)	0.4
eGFR, ml/min/1.73 m^2, median (IQR)	97.2 (88.8–103)	94.7 (74.2–100.3)	100.3 (89–103.4)	0.2

ALT, alanine aminotransferase; AST, aspartate aminotransferase; eGFR, estimated glomerular filtration rate; GGTP, γ-glutamyltransferase; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment, insulin resistance; LDL, low-density lipoprotein. Conversion factors to SI units are as follows: for cholesterol, 0.02586; creatinine, 88.4; bilirubin, 17.1; glucose, 0.05551; insulin, 6.945; and triglycerides, 0.0114.
A p value of <0.05 is considered statistically significant.
p values were calculated with t-test or the Mann-Whitney U test, as appropriate.

Table 3
The comparison of CAP and LS results between the baseline and after 3 and 5 months of therapy.

Variable	Baseline			*p1*	*p2*	*p3*	*p4*	*p5*	3 months *vs.* baseline				5 months *vs.* baseline				3 months *vs.* 5 months	
	Diet (D)	Metformin (M)	Control (C)	(D *vs.* M)	(C *vs.* M)	(C *vs.* D)	(D3 vs. M3)	(D5 vs. M5)	Diet (D3)	*p*	Metformin (M3)	*p*	Diet (D5)	*p*	Metformin (M5)	*p*	Diet *p*	Metformin *p*
CAP, dB/m	309 (270–323)	319 (302–342)	213 (175–229)	0.1	<0.01	<0.01	0.4	0.5	293 (274–323)	0.5	285 (249–311)	<0.01	278 (246–311)	0.3	295 (277–302)	0.002	0.3	0.9
LS, kPa	5.6 (4.4–6.8)	6.2 (4.8–7.7)	4.9 (4.3–5.4)	0.7	0.005	0.6	0.9	0.2	4.9 (4.4–7.2)	0.2	5.0 (4.2–8.8)	0.05	4.15 (3.8–7.8)	0.7	5.2 (4.3–6.8)	0.03	0.6	0.9

CAP, controlled attenuation parameter; LS, liver stiffness; D3 – dietary treatment alone after 3 months; D5 – dietary treatment after 5 months; M3 – metformin + dietary treatment after 3 months; M5 - metformin + dietary treatment after 5 months. A *p* value of <0.05 is considered statistically significant.
p values were calculated with Wilcoxon signed-rank test, *t*-test or the Mann-Whitney U test, as appropriate.

Table 4
Changes in biochemical parameters after 3 and 5 months of treatment.

Variable	Diet	Metformin	Diet				Metformin				Diet *vs.* metformin	
	Baseline		3 months (*vs.*baseline)		5 months (*vs.*baseline)		3 months (*vs.*baseline)		5 months (*vs.*baseline)		3 months	5 months
				p		*p*		*p*		*p*	*p*	*p*
Total cholesterol, mg/dl, mean (95% CI)	204.6 (188.66 – 220.5)	200.4 (178.7 – 222.2)	205.7 (187.9 – 223.5)	0.9	202.38 (172.7 – 232.1)	0.3	191.5 (171.2 – 211.7)	0.2	206.5 (170.9 –242.0)	0.3	0.1	0.1
HDL cholesterol, mg/dl, median (IQR)	47 (41 – 62)	42 (36 – 47)	49.0 (40.0 – 55.0)	0.1	46.2 (35.5 – 57.0)	0.03	41 (34 – 44)	0.4	40.8 (36.0 – 45.6)	0.7	0.06	0.4
LDL cholesterol, mg/dl, median (IQR)	127 (107 – 145)	109 (95.75 – 159.75)	133.0 (117.1 – 148.9)	0.7	127.9 (101.8 – 154.0)	0.7	117.6 (96.2 – 139.0)	0.08	114.1 (86.4 – 141.8)	0.7	0.1	0.4
Triglycerides, mg/dl, median (IQR)	104 (91 – 147)	152 (103 – 226)	110 (82 – 142)	0.8	94 (92 – 156)	0.3	155 (123 – 209)	0.5	187 (142 – 260)	0.4	0.03	0.01
AST, U/l, median (IQR)	22 (20 – 29)	26 (21 – 34)	22 (19 – 25)	0.3	16 (15 – 20)	0.006	24 (22 – 34)	0.9	25 (18 – 38)	0.4	0.3	0.07
ALT, U/l, median (IQR)	24.0 (14.75 – 33.25)	31 (23 – 53)	25 (18 – 30)	0.4	18 (12 – 22)	0.07	26 (18 – 37)	0.5	28 (19 – 50)	0.3	0.4	0.04
GGTP, U/l, median (IQR)	35 (25 – 38)	61 (26 – 78)	32 (23 – 39)	0.5	25 (18 – 32)	0.04	35 (21 – 69)	0.007	22 (21 – 69)	0.04	0.6	0.3
Bilirubin, mg/dl, median (IQR)	0.42 (0.34 – 0.57)	0.43 (0.28 – 0.52)	0.46 (0.30 – 0.55)	0.7	0.48 (0.39 – 0.64)	0.6	0.38 (0.33-0.59)	0.9	0.41 (0.37 – 0.50)	0.6	0.9	0.6
Glucose, mg/dl, mean (95% CI)	102.7 (96.0 – 109.4)	108.3 (101.8 – 114.8)	101 (93 – 111)	0.7	98 (92 – 114)	0.3	97 (91 – 109)	0.006	93 (92 – 98)	0.01	0.7	0.1
Insulin, μU/ml, median (IQR)	8.2 (6.2 – 11.2)	11.2 (8.2 – 13.2)	8.1 (5.5 – 12.9)	0.9	7.5 (6.1 – 16.0)	0.9	10.6 (8.6 – 12.3)	0.3	9.1 (7.2 – 14.5)	0.2	0.3	0.7
HOMA – IR, median (IQR)	2.40 (1.72 – 2.53)	2.71 (2.04 – 3.29)	2.06 (1.46 – 3.29)	<0.01	2.15 (1.66 – 4.42)	0.002	2.46 (1.93 – 3.0)	0.09	2.07 (1.51 – 3.29)	0.1	0.4	0.9
Creatinine, mg/dl, mean (95% CI)	0.84 (0.76 – 0.91)	0.78 (0.73 – 0.84)	0.80 (0.69 – 0.91)	0.3	0.78 (0.69 – 0.86)	0.05	0.72 (0.67 – 0.81)	0.006	0.78 (0.71 – 0.84)	0.3	0.2	0.8
eGFR, ml/min/1.73 m^2, median (IQR)	94.7 (74.2 – 100.3)	100.3 (89 – 103.4)	94.1 (83.7 – 103.9)	0.3	92.4 (85.4 – 98.5)	0.05	98.2 (91.5 – 106.0)	0.09	97.5 (88.7 – 106.3)	0.7	0.2	0.2

ALT, alanine aminotransferase; AST, aspartate aminotransferase; eGFR, estimated glomerular filtration rate; GGTP, γ-glutamyltransferase; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment – insulin resistance; LDL, low-density lipoprotein. Conversion factors to SI units are as follows: for cholesterol, 0.02586; creatinine, 88.4; bilirubin, 17.1; glucose, 0.05551; insulin, 6.945; and triglycerides, 0.0114.
A *p* value of <0.05 is considered statistically significant; *p* values were calculated with Wilcoxon signed-rank test.

Table 5
Changes in anthropometric parameters after 3 and 5 months of treatment.

Anthropometric parameter	Diet	Metformin	Diet				Metformin				Diet *vs.* metformin	
	Baseline		3 months (*vs.*baseline)		5 months (*vs.*baseline)		3 months (*vs.*baseline)		5 months (*vs.*baseline)		3 months	5 months
				p		*p*		*p*		*p*	*p*	*p*
Weight, kg, median (IQR)	82.9 (78.3 – 89.8)	88.2 (79.0 – 102.5)	80.7 (76.5 – 88.5)	0.3	81.9 (79.0 – 87.0)	0.04	86.0 (77.0 – 96.0)	<0.01	84.7 (81.0 – 92.5)	<0.01	0.4	0.4
BMI, kg/m^2, median (IQR)	29.2 (26.8 – 31.3)	31.4 (29.2 – 34.4)	29.1 (26.0 – 31.3)	0.4	28.7 (25.8 – 30.8)	0.04	29.7 (28.2 – 32.0)	<0.01	30.0 (28.5 – 31.9)	<0.01	0.4	0.2
Waist circumference, cm, median (IQR)	101.0 (96.0 – 106.0)	107.0 (102.0 – 118.0)	100.0 (98.0 – 105.0)	0.4	101.0 (95.8 – 107.3)	0.07	103.0 (99.0 – 108.0)	<0.01	102.0 (97.0 – 106.0)	<0.01	0.3	0.5
Hip circumference, cm, median (IQR)	104.0 (102.0 – 109.0)	112.0 (106.0 – 114.0)	104.0 (101.0 – 108.0)	0.8	104.5 (99.3 – 109.8)	0.1	108.0 (105.0 – 111.0)	<0.01	108.0 (105.0 – 116.0)	0.03	0.1	0.3
WHR, mean (95% CI)	0.96 (0.94 – 0.99)	0.97 (0.95 – 0.99)	0.96 (0.94 – 0.99)	0.9	0.97 (0.96 – 0.99)	0.8	0.97 (0.95 – 0.98)	0.4	0.96 (0.93 – 0.98)	0.2	0.9	0.9

BMI, body mass index; WHR, waist-to-hip ratio. A *p* value of <0.05 is considered statistically significant; *p* values were calculated with Wilcoxon signed-rank test.

ΔLS after 3 months of treatment: LS change was independently associated with metformin administration only (β = 0.31; *p* = 0.04).

ΔLS after 5 months of treatment: The univariate linear regression analysis demonstrated that LS change was associated with the baseline triglyceride concentration (β = -0.41; *p* = 0.03), initial ALT activity (β = -0.48; *p* = 0.01) and ALT activity after 3 months of treatment (β = -0.46; *p* = 0.02). In the multivariable regression analysis, the model which included the above depicted variables explained significantly ΔLS (R^2 = 0.31; *p* = 0.04). However, individually none of the variables correlated significantly with ΔLS.

Secondary outcome

The results of laboratory tests performed in both groups are presented in Table 4 and anthropometric data in Table 5. Contrary to dietary treatment alone, both 3- and 5-month diet and metformin therapy significantly improved almost all anthropometric parameters (weight, BMI, waist circumference and hip circumference), while dietary treatment resulted in significant reduction of weight, BMI and waist circumference after 5 months of treatment. Insulin resistance, as assessed by HOMA-IR was ameliorated in both groups, but a significant decrease was only found in patients on dietary treatment alone.

The 5-month dietary treatment resulted in a significantly decreased HDL-cholesterol concentration, metformin therapy did not influence on any of the lipid profile components.

No significant changes in serum ALT and AST activities were observed in the metformin group, while 5 months of dietary treatment alone resulted in a significant decrease in AST activity. Interestingly, both treatment protocols led to a significant reduction of GGTP activity.

Discussion

To our knowledge, there has been no study so far evaluating and comparing the effectiveness of metformin therapy using TE. In this prospective clinical trial, the results of transient elastography of the liver performed in patients with NAFLD and MetS indicate that 3- and 5-month metformin therapy results in the reduction of hepatic steatosis and fibrosis as expressed by CAP and LS, respectively. Furthermore, contrary to dietary treatment alone, combination of diet and metformin therapy appears to be superior to dietary treatment alone for improving anthropometric parameters.

Experimental studies revealed that metformin treatment in non-diabetic NAFLD models resulted in a significant reduction of hepatic steatosis and serum levels of ALT [13]. Metformin not only decreased steatosis and inflammation, but also ameliorated fibrosis in experimental NASH [14].

Several clinical trials have supported the beneficial potential of metformin in patients with NAFLD. Most of the studies have evaluated the effect of various doses of metformin on liver biochemistry (aminotransferase levels), histology and metabolic syndrome features, and reported an improvement in the indices of insulin resistance and reduction in liver function test values [15–17]. However, as regards histological improvement there is still a discrepancy between the promising results of metformin therapy observed by several researchers [15–18] and some recent findings indicating no impact of metformin treatment on hepatic steatosis, aminotransferases levels and insulin resistance markers as opposed to lifestyle changes [19–22].

Until now, there has only been one clinical study using CAP to evaluate the effects of pharmacotherapy on steatosis in NAFLD. Lee et al. [23] reported lobeglitazone ameliorated hepatic steatosis. However, a multivariable linear regression analysis demonstrated that patients treated with metformin and lobeglitazone exhibited a greater liver fat content reduction than patients treated with lobeglitazone alone [23].

It has been demonstrated that low-calorie diet and weight reduction [24], as well as pioglitazone and vitamin E treatment [25], rosiglitazone and metformin therapy [19] have decreased GGTP activity. No similar

results were reported in studies on metformin monotherapy in NAFLD, but there were few studies analyzing GGTP level variability during metformin treatment alone [17,18]. In this study, both metformin treatment and dietary intervention alone resulted in a significant reduction of GGTP levels, though the effects of metformin administration were observed at an earlier stage (after 3 months). It cannot be determined though whether GGTP decrease can be attributed to direct metformin influence or to weight reduction. Additive effect of both of these factors cannot be excluded.

No significant changes in ALT and AST activity were observed in patients treated with metformin, while dietary treatment alone did result in decrease of AST activity. However, the baseline aminotransferase activity was within reference range. The studies which demonstrated positive influence of metformin on aminotransferases in NAFLD comprised patients with the baseline elevation of liver enzyme activity [22,26,27]. The lack of changes in ALT and AST activity found in our study might have been due to a short observation period. Interestingly, the multiple regression analysis revealed that the rate of LS decrease was positively associated with the baseline ALT activity. This finding implies greater benefit of metformin treatment in patients with NASH.

The present study has certain drawbacks. The main limitation is a short treatment period. Therefore, no ultimate conclusions can be drawn regarding long-term effects of metformin administration on hepatic steatosis and stiffness, and laboratory findings. It must be also mentioned that groups differed initially in terms of age and triglyceridemia. Patients receiving metformin were significantly younger and had higher triglyceride level. Although, metformin administration did not influence triglyceridemia in this group of patients, it cannot be excluded that triglyceride level may influence response to the metformin treatment. Another limitation of the study is relatively small number of participants. In particular, the number of patients who underwent TE measurement after 5 months could contribute to additional interpretation bias. TE, which was conducted in this study, has not yet become a gold standard in diagnosis of NAFLD. However, a diagnostic accuracy of liver biopsy is limited by sampling variability and semiquantitative scale used for histological assessment [28]. An average biopsy specimen represents 1/50000 the size of the entire liver. Thus, the final diagnosis cannot be reliably made based on a single biopsy. The noninvasive nature of TE, along with measurement repeatability, make this tool a safe and reproducible alternative to a liver biopsy in monitoring of disease progression.

To sum up, we investigated the effects of metformin on MetS patients with NAFLD, using a transient liver elastography and gluco-metabolic profile analysis. Based on changes in elastographic parameters of the liver and the results of a multiple regression analysis, we found the combination of metformin and dietary treatment to be effective for the reduction of hepatic steatosis and fibrosis. However, considering the preliminary character of this study, its limitations, and inconsistent results of previous investigations in this area, it is impossible to draw conclusions on superiority of metformin therapy combined with diet over dietary treatment alone in patients with NAFLD. There is a need for further research on metformin efficacy in this group of patients.

Conflict of interest

Authors declare no conflict of interest

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