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Clinical trial: insulin-sensitizing agents may reduce consequences of insulin resistance in individuals with non-alcoholic steatohepatitis

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SUMMARY

Background
Currently, although only a few therapies normalize the liver test abnormalities with/without improving the liver histology, no pharmacologic therapy has proved to be effective for the treatment of non-alcoholic steatohepatitis.

Aim
To investigate the role of insulin sensitizers in the treatment of individuals with non-alcoholic steatohepatitis (NASH).

Methods
A total of 74 individuals with NASH (male/female, 44/30; mean age, 47.2 ± 9.0 years) were enrolled. Participants were divided into two distinct groups: group 1 (n = 25) participants were administered a conventional diet and exercise programme while those in group 2 (n = 49) were administered the diet and exercise programme plus insulin sensitizers.

Results
With respect to baseline metabolic, biochemical and histological parameters, no significant differences were observed between the two groups ($P > 0.05$). Insulin sensitizers significantly improved metabolic parameters (homeostasis model assessment-insulin resistance score, $P < 0.05$), serum aminotransferase levels [aspartate aminotransferase (AST): 45.9 ± 24.2 to 33.3 ± 17.7 IU/L, $P < 0.01$; alanine aminotransferase (ALT): 78.2 ± 46.3 to 47.3 ± 34.5 IU/L, $P < 0.001$] and histological features (median non-alcoholic fatty liver disease activity score: 5.0–3.0, $P = 0.01$), while diet and exercise improved serum aminotransferase levels (AST: 39.3 ± 11.1 to 30.0 ± 8.6 IU/L, $P < 0.01$; ALT: 66.9 ± 28.9 to 42.0 ± 16.2 IU/L, $P < 0.001$) at the end of the 48 weeks when compared to baseline. Insulin sensitizers improved the high-sensitivity C-reactive protein levels ($P < 0.01$). No serious adverse effects of insulin sensitizers were observed.

Conclusion
Insulin sensitizers can lead to improvement in metabolic, biochemical and histological abnormalities of NASH as a result of improved insulin sensitivity.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is characterized by hepatic steatosis in the absence of a history of significant alcohol use or other known liver disease.[1–5] NAFLD encompasses a histological spectrum that ranges from simple steatosis without concomitant inflammation or fibrosis to hepatic steatosis with a necroinflammatory component (steatohepatitis) that may or may not be associated with fibrosis.[2–5] NAFLD affects 10–24% of the general population in various countries and this prevalence increases to 57.5–74% in obese populations.[2, 3]

Non-alcoholic steatohepatitis (NASH) constitutes the subset of NAFLD most associated with progressive liver disease; it can cause cirrhosis in up to 20% of patients and liver-related death.[2–4, 6, 7] The pathogenesis of NASH is not well defined. The most widely supported theory is the 'two-hit' hypothesis[2, 3, 8–12] with insulin resistance (IR) leading to hepatic steatosis (first hit), and steatosis subsequently sensitizing the liver to a variety of metabolic injuries (second hit) leading to necroinflammation and fibrosis. NASH is associated commonly with obesity, diabetes mellitus, IR and hyperlipidaemia, as well as hypertension and hyperuricaemia. Thus, several investigators have suggested that NASH is the hepatic manifestation of the metabolic syndrome (MS).[2, 3, 5, 8–12]

Most patients with NASH have clinical and/or physiological evidence of IR.[3, 10–12] A major mechanism of IR is the down-regulation of insulin receptor substrate-1 (IRS-1) signalling by excess free fatty acids, which impair the tyrosine phosphorylation of IRS-1.[3, 12, 13] Impaired tyrosine phosphorylation, accelerated dephosphorylation and phosphorylation of serine residues have the effect of deactivating IRS-1, leading to IR.[3, 12, 13]

Although diet, exercise and weight loss ameliorate IR,[2, 3, 14, 15] individuals who fail to change their lifestyle require therapy to combat IR with insulin sensitizers such as biguanides and thiazolidinediones (TZDs). Metformin is the only currently available biguanide that is effective only in the presence of insulin and its major effects are to decrease hepatic glucose output and increase insulin action.[16] TZDs such as rosiglitazone are selective ligands of the nuclear transcription factor peroxisome proliferator-activated receptor-gamma (PPAR-γ).[17] They bind to and activate PPAR-γ, which facilitates increased insulin responsiveness, and reverse the down-regulation of IRS-1 by ameliorating IR in the liver.[17]

There is no proven beneficial therapy in NASH; diet and exercise remain the cornerstones. However, many individuals are unsuccessful in sustaining these lifestyle modifications. The aims of this study were to determine whether or not insulin sensitizers plus diet and exercise can improve the metabolic, biochemical and histological abnormalities in individuals with NASH compared with diet and exercise alone, and to investigate the tolerability of insulin sensitizers in such individuals.

MATERIAL AND METHODS

Patients

This was a prospective, longitudinal single centre study. A total of 129 individuals were newly diagnosed as NAFLD at Ankara University School of Medicine, Liver Diseases Outpatient Clinic, between December 2004 and October 2005. Of these, 74 individuals (male/female: 44/30, mean age: 47.2 $\pm$ 9.0 years) with NASH who were consecutively seen were enrolled into the study.

The diagnosis of NASH was based on biochemical, radiological and histological criteria. Criteria for inclusion were: (i) age >18 years; (ii) convincing evidence of absent or minimal alcohol consumption: <15 g alcohol/day for women and <20 g alcohol/day for men; (iii) absence of confounding disease including acute (hepatitis A, B or C) and/or chronic viral hepatitis; (iv) absence of heart and renal disease and (v) exclusion of other forms of liver disease including autoimmune, drug-induced and metabolic liver disease. The characteristics and demographics of the subjects are shown in Table 1. All individuals in this study signed informed consent before each procedure.

Biochemical tests

Fasting glucose, serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), bilirubin, cholesterol, and triglycerides levels and complete blood cell counts were measured by our central laboratory on a 24-channel automated chemical analyser using standard reagents. Insulin was measured by radioimmunoassay.

For exclusion of other forms of liver disease, serum iron, ferritin, copper and ceruloplasmin levels were measured, and serological studies for antinuclear

Table 1. Alteration in metabolic and biochemical parameters during the course of the study according to study group

	Diet + exercise (group 1)			Diet + exercise plus insulin sensitizer (group 2)		
	Baseline	At 48 weeks	*P*	Baseline	At 48 weeks	*P*
Age	45.8 ± 10.4	–		47.9 ± 8.3	–	
Gender (M/F)	9/16	–		21/27		
Weight (kg)	86.1 ± 12.3	85.4 ± 13.1	0.348	80.1 ± 11.7	77.8 ± 12.2	<0.001
BMI	32.2 ± 5.1	31.5 ± 5.3	0.002	31.2 ± 3.6	29.9 ± 3.4	<0.001
Body fat content (%)	33.4 ± 7.5	31.4 ± 8.3	0.019	32.7 ± 6.4	31.4 ± 6.9	0.008
Fasting plasma glucose (mg/dL) (*N*: 74–106 mg/dL)	99.5 ± 15.5	102.2 ± 55.2	>0.05	105.2 ± 19.1	93.5 ± 13.3	<0.001
Fasting plasma insulin (*N*: 2.1–22 μU/mL)	15.9 ± 13.3	16.3 ± 7.5	>0.05	19.8 ± 12.5	12.0 ± 9.0	<0.001
HOMA score	3.8 ± 3.1	3.9 ± 2.0	>0.05	5.3 ± 3.8	2.8 ± 2.4	<0.001
Cholesterol (mg/dL) (*N*: <200 mg/dL)	215.2 ± 30.9	195.4 ± 42.7	0.001	216.3 ± 52.3	204.4 ± 51.1	0.078
Triglycerides (mg/dL) (*N*: <150 mg/dL)	165.4 ± 64.1	151.6 ± 65.6	>0.05	202.4 ± 96.8	171.5 ± 85.5	0.018
WC	104.3 ± 8.5	101.9 ± 9.2	0.023	102.3 ± 8.5	97.8 ± 8.6	<0.001
HC	105.1 ± 10.2	104.3 ± 10.1	0.003	102.0 ± 6.3	100.7 ± 6.9	0.006
CRP (*N*: 0–3 mg/L)	3.3 ± 3.0	3.6 ± 3.9	>0.05	4.2 ± 3.1	2.3 ± 2.0	0.001
AST (IU/mL) (N: ≤37 IU/mL)	39.3 ± 11.1	30.0 ± 8.6	0.002	45.9 ± 24.2	33.3 ± 17.7	0.003
ALT (IU/mL) (N: ≤37 IU/mL)	66.9 ± 28.9	42.0 ± 16.2	<0.001	78.2 ± 46.3	47.3 ± 34.7	<0.001

Means ± s.d. are given.

antibody, antismooth muscle antibody and antimitochondrial antibodies were performed.

Histological assessments

Liver biopsies were performed before and at the end of the 48 weeks. The liver biopsy specimens were evaluated by one pathologist who was unaware of the subject's identity, group and all clinical information. Histological features of samples were interpreted as outlined by Brunt *et al.*[18] NAFLD activity score was calculated based on the criteria of Kleiner *et al.*[19]

Definition

The diagnosis of NASH was based on biochemical, radiological and histological criteria, requiring the presence of abnormal serum ALT levels; an abnormal ECHO pattern on sonography consistent with fatty infiltration[18, 20]; and a liver biopsy documenting steatosis and ballooning degeneration, with/without necroinflammatory activity, with/without portal inflammation, and with/without fibrosis or cirrhosis; and on exclusion of other forms of acute and chronic liver diseases.[18]

Diet and exercise

After diagnosis of NASH was confirmed, the management was focussed in the following areas: establishment of an appropriate diet and exercise programme including walking (initially as 300 steps/day for 3 days, thereafter adding 500 steps at 3-day intervals until a level of 10 000 steps was attained) and jogging (20 min twice a day),[21, 22] improvement in associated conditions such as moderate/severe hyperlipidaemia and discontinuation of potentially hepatotoxic drugs such as herbal medicine.

The subjects were randomly assigned (1:2) to receive diet and exercise or diet and exercise plus insulin sensitizers (either metformin or rosiglitazone) for 48 weeks. All subjects were divided basically into two distinct groups as follows: group 1 (n = 25) received a conventional diet of 25 kcal/kg × ideal body weight (kg) and an exercise programme. Three meals per day containing 60% carbohydrate, 25% fat and 15%

protein were provided for each individual. Group 2 (n = 49) received the diet and exercise programme plus metformin at a dose of 850 mg b.d. (group 2a, n = 24) or rosiglitazone at a dose of 8 mg daily (group 2b, n = 25). All subjects were interviewed by the research dietitian before the treatment, and the same diet and exercise programme was suggested. The dietitian and one of the authors monitored patient compliance with the diet and exercise programme during the 48 weeks and in the 6-month follow-up period via verbal communication.

IR was calculated on the basis of fasting plasma glucose and insulin values using the homeostasis model assessment-IR method [HOMA-IR: plasma glucose (mg/dL) × insulin (μU/mL)/405].[23] Body mass index (BMI) was calculated as weight in kilograms divided by height in metres squared. The percentage of body fat content (BFC%) was estimated by bioelectrical impedance analysis. Waist circumference (WC) was measured at the midpoint between the lower border of the rib cage and the iliac crest, and hip circumference (HC) was measured at the widest part of the hip region.

Follow-up

All subjects were seen at the fourth week and at 3-month intervals thereafter in the Outpatient Clinic. Vital signs, physical examination and compliance to diet and exercise programme were assessed. HOMA-IR score, BMI and BFC measurements were recorded. Blood samples were drawn for determining metabolic and biochemical parameters at diagnosis, at the fourth week and at 6-month intervals. Liver biopsies were performed at diagnosis and a second liver biopsy was offered at the end of the 48 weeks. During the 6-month follow-up period, all subjects in both groups were encouraged to continue their compliance with the diet and exercise programme.

Statistical analyses

All patients were included in the data analysis, with the exception of one receiving rosiglitazone who did not complete the study for personal reasons. Pearson chi-squared test was used to compare groups in terms of gender, and McNemar test was used for baseline and post-treatment ballooning necrosis scores. One-way analysis of variance was used for comparing groups in terms of age. Changes in continuous measurements between and within groups and baseline characteristics between groups were tested by mixed models (with Proc Mixed statement in SAS for Windows; SPSS Inc., Chicago, IL, USA). For non-normal values, Wilcoxon signed-rank test was used for groups 1 and 2 to compare baseline and post-treatment scores. Data were summarized by frequencies and percentages for categorical variables, mean ± s.d. and mean ± S.E. for continuous variables, and median (min, max) for non-normal values. For all tests, a two-tailed P-value of <0.05 was considered statistically significant. Analyses were performed with SPSS for Windows 11.5 and SAS for Windows V8 (for mixed models analysis, Proc Mixed was used).

RESULTS

Fifteen individuals with hyperlipidaemia (5 in group 1 and 10 in group 2) were on a lipid-reduced diet and antihyperlipidaemic agents. All of them continued such treatment.

With respect to baseline metabolic, biochemical and histological parameters, no significant differences were observed between the two groups (P > 0.05; Table 1).

Metabolic response

When compared to baseline, mean fasting plasma insulin level and HOMA-IR score had significantly decreased only in the treatment group (group 2) at the end of the 48 weeks (from 19.8 ± 12.5 to 12.0 ± 9.0 and 5.3 ± 3.8 to 2.8 ± 2.4 respectively; P < 0.001) (Figure 1a). However, mean BMI (from 32.2 ± 5.1 to 31.5 ± 5.3 and 31.2 ± 3.6 to 29.9 ± 3.4; P < 0.01, P < 0.001) (Figure 1b), BFC% (from 33.4 ± 7.5 to 31.4 ± 8.3 and 32.7 ± 6.4 to 31.4 ± 6.9; P = 0.02, P < 0.01), WC (from 104.3 ± 8.5 to 101.9 ± 9.2 cm and 102.3 ± 8.5 to 97.8 ± 8.6 cm; P = 0.02, P < 0.001) and HC (from 105.1 ± 10.2 to 104.3 ± 10.1 cm and 102.0 ± 6.3 to 100.7 ± 6.9 cm; P < 0.01, P < 0.01) significantly decreased in both groups 1 and 2 (Table 1).

Metformin (group 2a) had a significant effect on metabolic parameters (Table 2). Mean plasma insulin level (from 18.2 ± 12.2 to 12.0 ± 11.4, P = 0.02), HOMA-IR score (from 4.9 ± 3.9 to 2.8 ± 2.9, P < 0.01), BMI (from 30.8 ± 3.9 to 29.0 ± 3.5, P < 0.001), BFC% (from 31.4 ± 6.2 to 29.5 ± 6.9, P = 0.02), WC (from 101.9 ± 9.7 to 95.3 ± 8.9 cm, P < 0.001) and HC (from 100.9 ± 5.9 to 99.3 ± 7.4 cm, P = 0.04) were significantly reduced at the end of the 48 weeks.

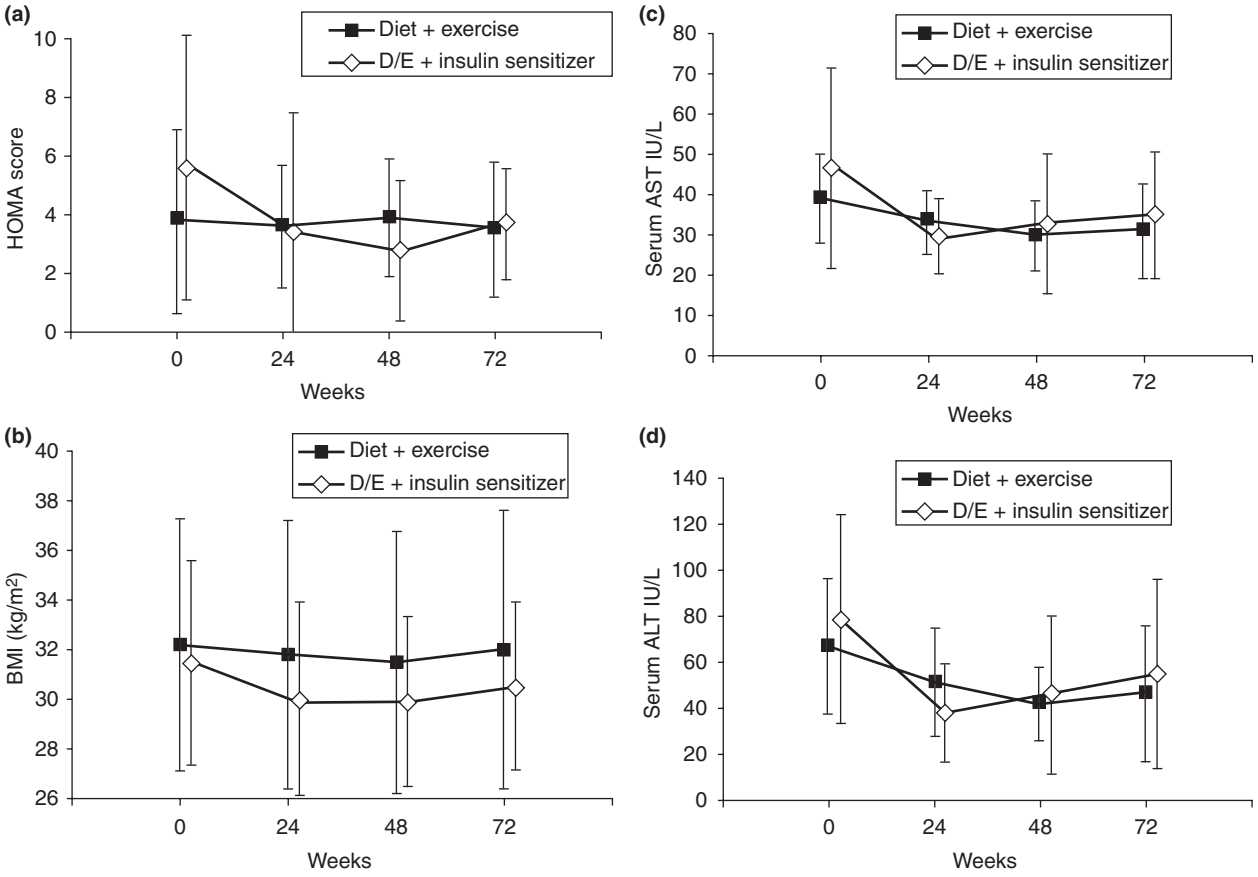

Figure 1. Alteration in metabolic (a: HOMA-IR score, b: BMI) and biochemical (c: serum AST levels, d: serum ALT levels) parameters during the course of the study. Means ± s.d. are given.

In the rosiglitazone group (group 2b), mean plasma insulin level (from 21.6 ± 12.8 to 12.0 ± 5.9, $P = 0.002$) and HOMA-IR score (from 5.7 ± 3.8 to 2.8 ± 1.8, $P = 0.001$) were significantly reduced at the end of the 48 weeks.

Insulin sensitizers significantly improved the high-sensitivity C-reactive protein (hs-CRP) levels (from 5.1 ± 4.3 to 2.3 ± 1.5 mg/L and 3.5 ± 1.2 to 2.4 ± 2.4 mg/L; $P < 0.01$ and $P = 0.04$ respectively), whereas hs-CRP levels remained unchanged in the diet and exercise group (from 3.3 ± 3.0 to 3.6 ± 3.9 mg/L, $P > 0.05$) at the end of the 48 weeks when compared to baseline.

Liver injury tests

Serum aminotransferases levels of both groups had significantly decreased at the end of the 48 weeks (serum AST level: from 39.3 ± 11.1 to 30.0 ± 8.6 IU/L and 45.9 ± 24.2 to 33.3 ± 17.7 IU/L; $P = 0.002$ and $P = 0.003$ respectively; serum ALT level: from 66.9 ± 28.9 to 42.0 ± 16.2 IU/L and 78.2 ± 46.3 to 47.3 ± 34.7 IU/L respectively; $P < 0.001$ for both) (Table 1). The improvement from baseline did not significantly differ between the two groups ($P > 0.05$).

At the end of the 48 weeks, serum AST levels had decreased to normal level in 88.0% (22/25) and 75.0% (36/48) of individuals in the two groups respectively (88.0% vs. 75.0%, $P > 0.05$), while serum ALT levels had decreased to normal level in 40.0% (10/25) and 62.5% (30/48) of individuals in the two groups respectively (40.0% vs. 62.5%, $P = 0.067$).

Histological response

Sequential liver biopsy at the end of the 48 weeks was performed in 29 individuals with NASH (8 in diet and exercise group; 21 in treatment group). NAS was significantly decreased only in the treatment groups at

Table 2. Alteration in metabolic and biochemical parameters during the course of the study according to insulin sensitizer

	Diet + exercise plus metformin group 2a			Diet + exercise plus rosiglitazone group 2b		
	Baseline	At 48 weeks	*P*	Baseline	At 48 weeks	*P*
BMI	30.8 ± 3.9	29.0 ± 3.5	<0.001	31.5 ± 3.4	30.9 ± 3.2	0.090
Body fat content (%)	31.4 ± 6.2	29.5 ± 6.9	0.017	34.0 ± 6.6	33.1 ± 6.7	0.205
Fasting plasma glucose (mg/dL) (*N*: 74–106 mg/dL)	105.5 ± 19.7	94.7 ± 12.9	0.005	105.0 ± 19.0	92.3 ± 13.8	<0.001
Fasting plasma insulin (*N*: 2.1–22 μU/mL)	18.2 ± 12.2	12.0 ± 11.4	0.023	21.6 ± 12.8	12.0 ± 5.9	0.002
HOMA score	4.9 ± 3.9	2.8 ± 2.9	0.002	5.7 ± 3.8	2.8 ± 1.8	0.001
Cholesterol (mg/dL) (*N*: <200 mg/dL)	229.4 ± 52.2	219.9 ± 55.4	0.287	203.2 ± 50.3	189.0 ± 42.3	0.171
Triglycerides (mg/dL) (*N*: <150 mg/dL)	235.9 ± 107.1	198.4 ± 94.3	0.082	168.9 ± 73.2	144.5 ± 67.6	0.120
WC	101.9 ± 9.7	95.3 ± 8.9	<0.001	102.7 ± 7.3	100.2 ± 7.7	0.061
HC	100.9 ± 5.9	99.3 ± 7.4	0.040	103.1 ± 6.8	102.1 ± 6.2	0.067
CRP (*N*: 0–3 mg/L)	5.1 ± 4.3	2.3 ± 1.5	0.005	3.5 ± 1.2	2.4 ± 2.4	0.042
AST (IU/mL) (*N*: ≤37 IU/mL)	49.7 ± 27.0	34.4 ± 21.6	0.047	42.2 ± 20.9	32.1 ± 13.2	0.018
ALT (IU/mL) (*N*: ≤37 IU/mL)	82.9 ± 52.9	50.0 ± 37.1	0.017	73.6 ± 39.3	44.6 ± 32.7	0.001

Means ± s.d. are given.

the end of the 48 weeks [from median 5.0 (range: 3–8) to 3.0 (range: 2–6), $P = 0.01$], while it remained unchanged in the diet and exercise group [from median 4.0 (range: 3–8) to 5.0 (range: 1–6), $P > 0.05$] (Table 3a).

In the treatment group, hepatic histological improvement was shown via a decrease in hepatic steatosis [from median 2.0 (range: 1–3) to 1.0 (range: 0–3), $P < 0.01$] and in ballooning [from median 2.0 (range: 1–2) to 1.0 (range: 1–2), $P = 0.02$]. Fibrosis improved in 4 patients (19.1%, 4/21) and stabilized in 11 patients (52.4%, 11/21). However, the change from baseline to the end of the 48 weeks was not significant ($P > 0.05$; Table 3b). In the diet and exercise group, no significant hepatic histological improvement from baseline to the end of the treatment was observed ($P > 0.05$; Table 3a).

Follow-up

Patient adherence to the diet and exercise programme in both groups was not sufficient and fell short of expectations, especially in the 6-month follow-up period, but there was no difference in compliance between the two groups based on information obtained via verbal communication.

The metabolic and biochemical parameters including BMI (from 31.5 ± 5.3 to 32.0 ± 5.6 and 29.9 ± 3.4 to 30.5 ± 3.4 respectively), fasting plasma glucose, insulin level, HOMA-IR score (from 3.9 ± 2.0 to 3.5 ± 2.3 and 2.8 ± 2.4 to 3.7 ± 2.0 respectively), BFC%, WC, serum cholesterol, triglycerides and aminotransferase levels (serum AST level: from 30.0 ± 8.6 to 31.3 ± 11.8 IU/L and 33.3 ± 17.7 to 35.2 ± 15.8 IU/L; serum ALT level: from 42.0 ± 16.2 to 46.5 ± 29.5 IU/L and 47.3 ± 34.7 to 55.2 ± 41.0 IU/L respectively) did not significantly change in either group during the 6-month follow-up period (Figure 1). During the study period, diabetes mellitus developed in eight patients (two in group 1 and six in group 2).

Adverse events

All individuals with NASH completed the study with the exception of one subject who received rosiglitazone and who cited personal reasons.

No serious adverse event associated with insulin sensitizers was observed. Rosiglitazone did not

Table 3. Sequential follow-up of liver histology

	Diet + exercise (group 1), $n = 8$		Diet + exercise plus treatment (group 2), $n = 21$	
	Baseline	At 48 weeks	Baseline	At 48 weeks
(a)				
Steatosis	2 (1–3)	1.5 (0–3)	2 (1–3)	1* (0–3)
Lobular inflammation	1 (0–3)	1 (0–2)	1 (0–3)	1 (0–3)
Ballooning	2 (1–2)	2 (1–2)	2 (1–2)	1* (1–2)
Portal inflammation	1 (0–1)	0 (0–1)	0 (0–2)	1 (0–1)
Fibrosis	0 (0–4)	0 (0–2)	0 (0–2)	0 (0–2)
NAS score	4 (3–8)	5 (1–6)	5 (3–8)	3* (2–6)
Brunt's grade	1.5 (1–3)	1.5 (0–2)	2 (1–3)	1 (1–2)
	Diet + exercise + metformin, $n = 10$		Diet + exercise + rosiglitazone, $n = 11$	
	Baseline	At 48 weeks	Baseline	At 48 weeks
(b)				
Steatosis	2.5 (1–3)	1.5 (1–3)	2 (1–3)	1* (0–3)
Lobular inflammation	1 (1–3)	1 (0–2)	1 (0–2)	1 (0–3)
Ballooning	2 (1–2)	2 (1–2)	2 (1–2)	1 (1–2)
Portal inflammation	0 (0–1)	0.5 (0–1)	0 (0–2)	1 (0–1)
Fibrosis	0 (0–2)	0 (0–1)	0 (0–1)	1 (0–2)
NAS score	5 (3–8)	4 (3–6)	5 (3–6)	3 (2–6)
Brunt's grade	1.5 (1–3)	1 (1–2)	2 (1–2)	1 (1–2)

Medians (range) are given.
NAS, non-alcoholic fatty liver disease activity score.
* From baseline to the end of the 48 weeks, $P < 0.05$.

significantly increase the BMI, WC or HC at the end of the 48 weeks compared to baseline ($P > 0.05$; Table 1).

DISCUSSION

IR is the most specific metabolic risk and pathophysiological feature of NASH.[2, 3, 5, 10, 12] As IR may be a significant causal factor in the development of NASH,[2, 3, 5, 10, 12] therapeutic interventions aimed at improving insulin sensitivity may be one of the cornerstone approaches to treatment. In several previous studies,[24–27] either metformin or TZDs added to a caloric-restricted diet significantly improved IR and abnormal biochemical parameters; however, there is no clear consensus on histological improvement.[24–26] Recently, Belfort *et al.*[27] treated 26 individuals with NASH with a hypocaloric diet and pioglitazone for 24 weeks. The investigators concluded that diet plus pioglitazone led to metabolic, biochemical and histological improvement in individuals with NASH.[27] However, based on the results of the previous studies, larger controlled clinical trials of longer duration are warranted to determine the long-term clinical benefit and safety of insulin sensitizers in NASH.

In this prospective, longitudinal controlled study, a total of 74 individuals with NASH were enrolled. Among them, 49 were treated with insulin sensitizers (metformin or rosiglitazone) for 48 weeks. At the end of 48 weeks, both insulin sensitizers had improved insulin sensitivity as a result of their lowering effect on fasting plasma insulin levels and HOMA-IR score. Metformin also significantly improved BMI, BFC%, WC and HC in such individuals.

In addition to metabolic improvement, insulin sensitizers improved the abnormal serum aminotransferases levels in individuals with NASH. From baseline to the

end of the 48 weeks, serum aminotransferase levels in both treatment groups had significantly decreased. This improvement continued during the 6-month follow-up period.

In this study, in contrast to the diet and exercise group, a significant improvement in histological features was also observed in the treatment group. The histological features of steatohepatitis, including steatosis and ballooning, were reduced only in the treatment group. However, hepatic fibrosis did not significantly change in either group. This finding is comparable with that reported by Belfort *et al.*[27] This indicates that insulin-sensitizing agents probably reduce hepatic lipid synthesis as a result of amelioration of insulin sensitivity in hepatic tissue in individuals with NASH.

Another important issue is whether or not the beneficial effects of the insulin-sensitizing agents on the metabolic, biochemical and histological parameters can be sustained in the long-term. Neuschwander-Tetri *et al.*[25] observed that the liver enzymes and glycaemic control reverted to pre-treatment values 6 months after discontinuation of rosiglitazone. The investigators suggested that the beneficial effects of insulin sensitizers may last only as long as drug intervention is used.[25] In this study, no reversal of any of the metabolic or biochemical beneficial effects of the insulin sensitizers was observed during the 6-month follow-up period. As several investigators have suggested that NASH is the hepatic manifestation of the MS, which requires life-time intervention,[2, 3, 5, 10, 12] we suggest that individuals with NASH associated with IR should be treated with dietary intervention plus insulin sensitizers as long as possible.

Several investigators reported that individuals with NAFLD have significantly higher serum levels of markers of inflammation such as CRP, interleukin-6, tumour necrosis factor-α and other proinflammatory cytokines compared with healthy subjects.[27–29] Targher[29] compared serum levels of hs-CRP in 85 individuals with NAFLD. The investigators found that individuals with NAFLD have a marked increase in serum hs-CRP levels compared with matched control subjects, and that serum hs-CRP levels are significantly higher in individuals with NASH than in those with hepatic steatosis.[29] In this study, mean baseline serum hs-CRP levels in each group were higher than normal levels; both insulin sensitizers significantly improved hs-CRP levels. This finding is the first observation in the literature and indicates that individuals with NASH are characterized by a low-grade systemic inflammation, which insulin sensitizers seem to reduce.

The use of TZDs is associated with weight gain, fluid retention, anaemia, cardiac failure and hepatotoxicity.[17] In this study, no clinically significant adverse events related with either metformin or rosiglitazone were observed, although one subject who received rosiglitazone did not complete the study for personal reasons. A major limitation of rosiglitazone therapy has been reported as weight gain, which might eventually reverse any beneficial effect on steatosis. In this study, there was no significant change in terms of BMI, WC or HC in the rosiglitazone group from baseline to the end of the 48 weeks, or 6 months after the treatment was discontinued. Thus, it seems that insulin sensitizers are safe and tolerable in NASH.

One limitation of this study is that only 41% of the participants accepted to undergo the second liver biopsy, which was offered at the end of the 48 weeks to evaluate histological improvement. Others refused the second liver biopsy for reasons of their having normal liver tests.

In conclusion, according to the results of this study, insulin sensitizers (metformin or rosiglitazone) can lead to an improvement in metabolic, biochemical and histological parameters in NASH as a result of improved insulin sensitivity reflected in lowering of the abnormal HOMA-IR scores. These data suggest that these agents are safe and tolerable in such individuals.

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