

ORIGINAL ARTICLE

Effect of multifactorial treatment on non-alcoholic fatty liver disease in metabolic syndrome: a randomised study

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Key words: Atorvastatin – Dyslipidaemia – Fenofibrate – Metabolic syndrome – Non-alcoholic fatty liver disease – Orlistat – Weight loss

ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) is the hepatic manifestation of the metabolic syndrome (MetS). There is no established treatment for NAFLD.

Aim: To evaluate a multifactorial intervention in the treatment of NAFLD.

Methods: A prospective, open-label, randomised study in non-diabetic patients ($n = 186$) with

MetS (follow-up: 54 weeks). All patients had both biochemical and ultrasonographic evidence of NAFLD at baseline. Other causes of liver disease were excluded. Patients received lifestyle advice and treatment for hypertension (mainly inhibitors of the renin-angiotensin system), impaired fasting glucose (metformin), obesity (orlistat) and dyslipidaemia [randomly allocated to atorvastatin

20 mg/day ($n = 63$) or micronised fenofibrate 200 mg/day ($n = 62$) or both drugs ($n = 61$)). Liver ultrasonography was assessed at baseline and at the end of the study.

Results: At the end of treatment, 67% of patients on atorvastatin, 42% on fenofibrate and 70% on combination treatment no longer had biochemical plus ultrasonographic evidence of NAFLD ($p < 0.05$ vs. baseline for all comparisons). The percentage of patients who no longer had evidence of NAFLD was significantly higher ($p < 0.009$) in the atorvastatin and combination groups compared with the

fenofibrate group. This effect was independently related to drug treatment, as well as to reductions in high-sensitivity C-reactive protein, waist circumference, body weight, triglycerides, low-density lipoprotein-cholesterol, total cholesterol, systolic blood pressure and glucose. Four patients discontinued treatment because of adverse effects.

Conclusions: Multifactorial intervention in MetS patients with both biochemical and ultrasonographic evidence of NAFLD offsets surrogate markers of NAFLD (i.e. elevated aminotransferase plus echogenic liver).

Introduction

Non-alcoholic fatty liver disease (NAFLD) describes a common clinicopathological condition characterised by hepatic lipid deposition in patients without a history of excessive alcohol ingestion¹. The term NAFLD incorporates a spectrum of histological findings, varying from steatosis alone to steatosis with inflammation, necrosis, fibrosis or cirrhosis². The prevalence of NAFLD in the general population ranges from 10 to 39%^{1,2}. Because of the increasing prevalence of obesity³, the incidence of NAFLD is likely to increase¹. The clinical implications of this condition are still unclear, but NAFLD may progress to more serious liver disease and require liver transplantation².

Insulin resistance [the cornerstone abnormality of the metabolic syndrome (MetS)] was the laboratory finding most closely associated with the presence of NAFLD in a large series of patients⁴. Accordingly, NAFLD might represent another feature of the MetS, as defined by the National Cholesterol Educational Program (NCEP) Adult Treatment Panel (ATP) III⁵, with decreased insulin sensitivity being a common factor⁴. The strong association of NAFLD with other features of the MetS such as obesity, central fat distribution, diabetes mellitus (DM), dyslipidaemia, hypertension and atherosclerotic cardiovascular disease (CVD) supports this hypothesis⁶.

We recently reported that multi-targeted treatment of the MetS results in a significant reduction in the estimated 10-year risk for coronary heart disease⁷. This treatment was associated with a significant decrease in the number of patients with MetS and improved lipid variables. However, evidence regarding treatment efficacy of NAFLD is rather scarce¹⁻⁴. Thus, we undertook the present prospective, open-labelled, randomised study in non-diabetic patients with MetS who also had evidence of NAFLD (defined as abnormal serum aminotransferase activity plus echogenic liver). We aimed to evaluate if multi-targeted treatment of MetS can both safely and effectively improve the biochemical and ultrasonographic features of NAFLD.

Patients and methods

Participants

This prospective, open-labelled, randomised, intention-to-treat study was carried out in the Department of Internal Medicine of the Hippocraton Hospital, Aristotelian University, Thessaloniki, Greece, and included 186 consecutive subjects with MetS plus biochemical (elevated aminotransferase activity) and ultrasonographic (echogenic liver) evidence of NAFLD. Recruitment started in September 2003 and was concluded within a 5-month period. Follow-up was completed by January 2005. The patients were free of DM (i.e. fasting glucose levels < 7 mmol/L; 126 mg/dL) and CVD (diagnosed on the basis of personal history, clinical examination and non-invasive methods). The inclusion criteria were: (a) the presence of the MetS (NCEP ATP III definition⁵), (b) low density lipoprotein cholesterol (LDL-C) > 3.4 mmol/L (130 mg/dL), (c) ultrasonographic evidence of fatty liver and, (d) elevated serum aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) activity.

The exclusion criteria were: (a) history of excessive alcohol ingestion (> 20 g/day), (b) chronic viral (B or C) or autoimmune hepatitis, (c) copper storage disease, (d) impaired renal function (serum creatinine > 115 μ mol/L; 1.5 mg/dL), (e) serum triglyceride (TG) levels > 500 mg/dL (5.6 mmol/L) and (f) aminotransferase or creatine kinase activity > 3 -fold and 5-fold the upper limit of normal (ULN), respectively.

The present study ($n = 186$) is a subgroup analysis of a larger study ($n = 300$), where we assessed the effects of the same treatment on cardiovascular risk, prevalence of MetS and several vascular risk factors⁷. In the present study, we focus on the patients who had both elevated serum aminotransferase activity and an echogenic liver at baseline. This was a pre-specified subanalysis as per protocol. The details of each treatment subgroup are given below (treatment of dyslipidaemia subsection).

The study received ethical approval and informed consent was obtained from all subjects before enrolment.

Definition of the MetS

Participants having three or more of the following criteria (according to the NCEP ATP III report)⁵, were defined as having the MetS:

1. Abdominal obesity: waist circumference > 102 cm in men and > 88 cm in women.
2. High TGs: ≥ 1.7 mmol/L (150 mg/dL).
3. Low high density lipoprotein cholesterol (HDL-C) level: < 1.0 mmol/L (40 mg/dL) in men and < 1.3 mmol/L (50 mg/dL) in women.
4. High blood pressure (BP): $\geq 130/85$ mmHg or use of antihypertensive medication.
5. Impaired fasting glucose (IFG): ≥ 6.1 mmol/L (110 mg/dL).

Study protocol

Lifestyle advice: all subjects received lifestyle advice. This included exercise (walking for at least 30 min, 5 days a week or equivalent exercise) and a low-fat (NCEP ATP III)⁵ low-calorie diet. After estimating the appropriate caloric intake for a specific subject (according to his/her job and leisure time activity) we provided (according to the suggestions of a dietician) a computer-generated diet (taking into consideration dietary preferences). The recommended daily caloric intake was 500 calories less than that estimated as appropriate. Compliance was established at every visit with a 3-day food intake questionnaire. The participants did not change their drinking habits during the study.

Treatment of arterial hypertension: hypertension was treated in a stepwise manner with angiotensin converting enzyme inhibitors (ACEIs) or angiotensin II receptor blockers (ARBs). If these did not achieve a target of 130/85 mmHg, a low dose (12.5 mg/day) of hydrochlorothiazide was added. If another antihypertensive was required, a calcium channel blocker was used. Finally, another antihypertensive agent was added, if necessary, but the intention was to avoid metabolic side effects of antihypertensive drug treatment (e.g. high doses of diuretics and beta-blockers).

Treatment of obesity: in patients with a body mass index (BMI) > 30 kg/m², orlistat (120 mg three times a day) was prescribed. Patients with a BMI of 25–30 kg/m² received only lifestyle advice.

Treatment of IFG: IFG was treated with metformin. The starting dose was 850 mg/day and was increased to 850 mg twice daily if fasting glucose levels remained > 6.1 mmol/L (110 mg/dL).

Treatment of dyslipidaemia: subjects were randomly allocated to three treatment groups in the main study⁷. Each treatment group initially included 100 patients with the same inclusion and exclusion criteria (see

above)⁷. After excluding those patients who did not have both elevated serum aminotransferase activity and an echogenic liver at baseline, the first group consisted of 63 patients who received atorvastatin 20 mg/day, the second consisted of 62 patients who received micronised fenofibrate 200 mg/day and the third group received both drugs and included 61 patients.

In the initial study⁷ randomisation was performed with the Random Number Generation Computer Program and numbers were uncovered at enrolment. The randomisation sequence was generated by AAP, the enrolment was performed by OIG, OIK and KG and the random allocation was performed by VGA, who was blinded to hypolipidaemic drug treatment.

Endpoints

The primary endpoint of this study was to assess efficacy of multifactorial treatment of MetS in reducing the prevalence of both biochemical and ultrasonographic evidence of NAFLD within a 54-week period. The secondary endpoint was to assess the safety of such intervention.

Laboratory assessments

After an overnight fast, total cholesterol (TC), HDL-C, TG, serum uric acid (SUA), AST (ULN = 35 U/L), ALT (ULN = 40 U/L), gamma-glutamyl transpeptidase (G-GT, UNL = 45 U/L) and alkaline phosphatase (AP, ULN = 170 U/L) were assessed using an Olympus AU 560 autoanalyser and respective reagents (Olympus Diagnostica GmbH, Co. Clare, Ireland). LDL-C was calculated by the Friedewald formula [LDL-C in mg/dL = TC in mg/dL – (TG/5 in mg/dL + HDL-C in mg/dL)]. Non-HDL-C was calculated by subtracting the HDL-C value from that of TC. Fasting blood glucose was measured using an Olympus AU 560 autoanalyser and respective reagents (Medicon Hellas, Athens, Greece). Serum creatinine (SCr) was measured using the Jaffé method [reference range 55–115 μ mol/L (0.6–1.5 mg/dL)], and SUA with an enzymatic colorimetric test [(uricase); reference range 150–415 μ mol/L (2.5–7.0 mg/dL)]. SCr levels were adjusted for age, gender and BMI. Plasma fibrinogen was measured by the Clauss method, using the Fibrindex test (Ortho Diagnostic, Raritan, NJ, USA). High-sensitivity C-reactive protein (hsCRP) was measured using a standardised ELISA assay (Alpha Diagnostic International, San Antonio, Texas, USA (reference range: 0–1 mg/L).

Physical examination and assessment of lipid profile, liver function tests and fasting blood glucose were performed every 6 weeks, while plasma fibrinogen, SCr, SUA and hsCRP were assessed at baseline and at the end of the study.

Liver ultrasonography

This method is sensitive in detecting fatty liver, non-invasive, relatively easy to perform and ensures patient compliance⁸. Liver ultrasonography was performed by two highly trained specialists who were blinded to treatment and to the same subject's baseline ultrasound (i.e. they did not have access to the report they had made a year earlier). The assessment of liver ultrasound was qualitative; a 3-point scale was used. Specifically, liver echogenicity was classified as normal, increased (diffuse increase in fine echoes in liver parenchyma with normal or slightly impaired visualisation of intrahepatic vessels and diaphragm) or markedly increased (marked increase in fine echoes with poor or non-visualisation of the intrahepatic vessel borders diaphragm and posterior right lobe of the liver)⁹. By definition, those with normal echogenicity were not included in this study. Both specialists had to agree on the echogenic classification. Coefficients (κ) were calculated to assess the degree of agreement between the two radiologists on the severity of liver echogenicity. Interobserver agreement for severity of steatosis was substantial ($\kappa = 0.78$, 95% confidence interval 0.51–0.93). In cases of disagreement, a decision was taken by consensus agreement between the two examiners.

Statistical analyses

An intention-to-treat analysis of all patients randomised to the three treatment groups was performed. Clinical and laboratory findings within groups were compared by unpaired Student's *t*-tests for parametric data and

by chi square-tests for categorical data. Parametric data within and between the three groups were compared using analysis of variance (ANOVA), while non-parametric data were compared using the Kruskal–Wallis test. Univariate analysis was used to investigate which factors predicted response to treatment, i.e. the absence of both biochemical and ultrasonographic indices of NAFLD at the end of the study, in the whole study population. All significant predictors of treatment response in the univariate analysis were entered into a multivariate model. Furthermore, the effect of drug treatment (other than hypolipidaemic medication) was separately examined in another multivariate model. A confidence interval of 95% was used and a two-tailed $p < 0.05$ was considered significant. All analyses were carried out using the SPSS 12.00 software package (SPSS, Inc., Chicago, IL). Because this was an intention-to-treat study all patients were included in the analysis.

Results

Characteristics of the study population are shown in Table 1 and baseline lipid values as well as liver function tests in Table 2. From all the 186 patients included in the study, 154 had increased and 32 had markedly increased liver echogenicity. There were no significant differences in echogenic severity distribution between the three treatment groups at baseline.

Medications used in the study population are listed in Table 3.

Table 1. Baseline demographic and clinical characteristics of the three treatment groups*

	Atorvastatin, 20 mg/day (n = 63)	Fenofibrate, 200 mg/day (n = 62)	Combination treatment (n = 61)
Age (years)	60 ± 11	61 ± 12	59 ± 13
Gender male (%)	65	63	66
BMI (kg/m ²)	32 ± 7	32 ± 5	33 ± 9
Current smokers (%)	38	36	38
Ex smokers (%)	22	25	24
Never smoked (%)	40	39	38
IFG (%)	47	46	48
Elevated BP (%)	83	81	84
High TG (%)	68	66	68
Low HDL-C (%)	56	56	57
Central obesity (%)	91	89	92

Age in years and BMI (body mass index) in kg/m² are expressed in mean values ± SD

IFG = impaired fasting venous plasma glucose levels > 6.0 mmol/L (110 mg/dL), elevated BP = blood pressure ≥ 130/85 mmHg, high TG = triglycerides > 1.7 mmol/L (150 mg/dL), low HDL-C = high density lipoprotein cholesterol < 1.0 mmol/L (40 mg/dL) for men and < 1.3 mmol/L (50 mg/dL) for women, central obesity = waist circumference > 102 cm for men and > 88 cm for women, MetS = metabolic syndrome (≥ 3 of IFG, elevated BP, high TG, low HDL-C, central obesity)

*There were no significant differences between the three groups

Table 2. Baseline clinical and laboratory variables in the three treatment groups*

All patients	Atorvastatin, 20 mg/day (n = 63)	Fenofibrate, 200 mg/day (n = 62)	Combination treatment (n = 61)
TC (mmol/L)	6.1 ± 1.1	6.0 ± 1.0	6.2 ± 1.0
LDL-C (mmol/L)	4.0 ± 1.0	3.9 ± 1.2	4.1 ± 1.1
HDL-C (mmol/L)	1.0 ± 0.4	1.1 ± 0.3	1.1 ± 0.5
Non-HDL-C (mmol/L)	5.1 ± 0.8	4.9 ± 0.9	5.1 ± 1.0
TG (mmol/L)	2.3 ± 1.2	2.2 ± 1.1	2.4 ± 1.3
Fasting glucose (mmol/L)	5.9 ± 1.0	5.8 ± 1.0	5.8 ± 0.9
SCr (µmol/L)	94 ± 14	93 ± 12	95 ± 15
SUA (µmol/L)	491 ± 70	495 ± 73	488 ± 75
SBP (mm Hg)	147 ± 10	145 ± 14	146 ± 13
DBP (mm Hg)	91 ± 7	89 ± 6	90 ± 9
hsCRP (mg/L)	4.5 ± 0.9	4.4 ± 0.8	4.7 ± 1.0
Fibrinogen (mg/dL)	424 ± 45	416 ± 52	418 ± 46
Waist circumference (cm)	108 ± 13	107 ± 12	109 ± 14
Body weight (kg)	95 ± 15	96 ± 14	96 ± 11
ALT (U/L)	54 ± 23	52 ± 25	51 ± 24
AST (U/L)	38 ± 11	39 ± 10	38 ± 13
Gamma-GT (U/L)	52 ± 28	54 ± 26	55 ± 29
AP (U/L)	110 ± 43	108 ± 45	111 ± 39

TC = total cholesterol, LDL-C = low density lipoprotein cholesterol, HDL-C = high density lipoprotein cholesterol, TG = triglycerides, SCr = serum creatinine, SUA = serum uric acid, AST = aspartate aminotransferase, ALT = alanine aminotransferase, AP = alkaline phosphatase, gamma-GT = gamma-glutamyl transpeptidase, hsCRP = high sensitivity C reactive protein, SBP = systolic blood pressure, DBP = diastolic blood pressure, MetS = metabolic syndrome

To convert data from mmol/L to mg/dL multiply plasma glucose by 18, total, LDL, HDL and non-HDL cholesterol values by 38.7 and triglycerides by 88.6. To convert SCr and SUA values from µmol/L to mg/dL divide by 76.29 and 59.48, respectively

*There were no significant differences between the three groups. Values are expressed as mean ± SD

Table 3. Medications (other than hypolipidaemic) used in the study population*

	Atorvastatin 20 mg/day group (n = 63)	Fenofibrate 200 mg/day group (n = 62)	Combination group (n = 61)
Metformin (%)	55	56	55
ACEIs (%)	60	61	63
ARBs (%)	3	4	3
Hydrochlorothiazide (%)	46	44	43
CCBs (%)	41	43	42
Other antihypertensive drugs† (%)	12	14	15
Orlistat (%)	39	38	40

ACEIs = angiotensin converting enzyme inhibitors, ARBs = angiotensin receptor blockers, CCBs = calcium channel blockers, †beta-blockers, alpha-blockers or centrally-acting agents

*There were no significant differences between the three groups

Laboratory and clinical parameters [Tables 2 (baseline) and 4 (after 54 weeks of treatment)]

The lipid profile significantly improved in all three treatment groups. Waist circumference, systolic BP, diastolic BP, fasting blood glucose and SUA were significantly reduced in all three treatment groups (Table 4). SCr was reduced in the atorvastatin group, remained unchanged in the combination group and increased in the fenofibrate group (Table 4).

Liver function tests and ultrasound

At the end of the study, 67% in the atorvastatin, 42% in the fenofibrate and 70% in the combination treatment groups no longer had any surrogate evidence of NAFLD (as established by normalisation of serum aminotransferase plus a normal liver echopattern) ($p < 0.05$ vs. baseline for all comparisons) (Table 4). The percentage of patients who no longer had any surrogate evidence of NAFLD was significantly higher in the atorvastatin and the combination groups compared with the

Table 4. Clinical and laboratory parameters at the end of the study. Absolute values with percentage change in parenthesis

	Atorvastatin 20 mg/day (n = 63)	Fenofibrate 200 mg/day (n = 62)	Combination treatment (n = 61)
TC (mmol/L)	4.2 ± 0.8 (-31)*	5.3 ± 0.9 (-12)*†	4.1 ± 0.7 (-34)*
LDL-C (mmol/L)	2.6 ± 0.6 (-35)*	3.5 ± 0.5 (-11)*†	2.4 ± 0.4 (-39)*
HDL-C (mmol/L)	1.1 ± 0.5 (9)*	1.3 ± 0.3 (20)*	1.4 ± 0.3 (24)*
Non-HDL-C (mmol/L)	3.2 ± 0.8 (-38)*	4.0 ± 0.7 (-20)*†	2.7 ± 0.5 (-47)*
TG (mmol/L)	1.6 ± 0.7 (-32)*	1.3 ± 0.4 (-41)*	1.2 ± 0.4 (-48)*
Fasting glucose (mmol/L)	5.0 ± 0.6 (-15)*	4.9 ± 0.5 (-15)*	4.9 ± 0.4 (-15)*
SCr (µmol/L)	82 ± 8 (-13)*	104 ± 15 (8)*†	90 ± 10 (-4)
SUA (µmol/L)	432 ± 44 (-12)*	416 ± 52 (-16)*	405 ± 42 (-17)*
SBP (mmHg)	123 ± 7 (-16)*	125 ± 6 (-14)*	120 ± 5 (-18)*
DBP (mmHg)	77 ± 3 (-15)*	76 ± 4 (-15)*	75 ± 4 (-17)*
hsCRP (mg/L)	1.5 ± 0.7 (-66)*	2.5 ± 0.6 (-43)*	1.4 ± 0.4 (-69)*
Fibrinogen (mg/dL)	403 ± 38 (-5)*	345 ± 30 (-17)*	343 ± 25 (-18)*
Waist circumference (cm)	98 ± 6(-9)*	96 ± 7 (-10)*	98 ± 9 (-10)*
Body weight (kg)	83 ± 7 (-12)*	84 ± 6 (-13)*	85 ± 7 (-11)*
ALT (U/L)	32 ± 9 (-40)*	36 ± 11 (-31)*	30 ± 7 (-41)*
AST (U/L)	25 ± 8 (-34)*	27 ± 9 (-30)*	24 ± 6 (-37)*
Gamma-GT (U/L)	33 ± 8 (-37)*	37 ± 18 (-31)*	32 ± 9 (-42)*
AP (U/L)	75 ± 12 (-32)*	78 ± 16 (-28)*	69 ± 9 (-38)*
Biochemical + ultrasonographic evidence of NAFLD (n)	21 (-67)*	36 (-42)*†	18 (-70)*
MetS (n)	16 (-75)*	15 (-76)*	24 (-61)*

Abbreviations as in Table 2

* = $p < 0.05$ vs. baseline, † = $p < 0.05$ vs. the other treatment groups

NAFLD = non-alcoholic fatty liver disease. Values are expressed as mean ± SD

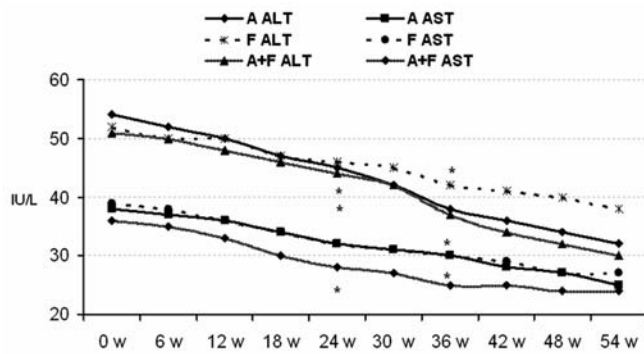


Figure 1. Time course of the change in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels during the 54 week treatment period with atorvastatin (A, n = 63), fenofibrate (F, n = 62) and their combination (A + F, n = 61) in non-diabetic subjects with the metabolic syndrome and both biochemical and ultrasonographic evidence of NAFLD. *Indicates the time point after which the changes became statistically significant ($p < 0.05$)

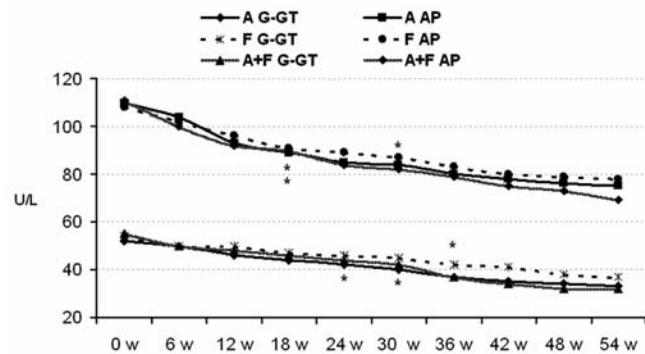


Figure 2. Time course of the change in gamma-glutamyl transpeptidase (G-GT) and alkaline phosphatase (AP) levels during the 54 week treatment period with atorvastatin (A, n = 63), fenofibrate (F, n = 62) and their combination (A + F, n = 61) in non-diabetic subjects with the metabolic syndrome and both biochemical and ultrasonographic evidence of NAFLD. *Indicates the time point after which the changes became statistically significant ($p < 0.05$)

fenofibrate group (Table 4, $p < 0.009$). In contrast, the percentage of patients no longer having MetS at the end of study was similar in all three treatment groups (Table 4).

In addition, 11.1% of patients in the atorvastatin group, 14.5% in the fenofibrate group and 8.2% in the combination group still had echogenic liver at the end of the study but with normal aminotransferase activity.

No patient in any group had elevated aminotransferase activity at the end of the study without an echogenic liver. All these patients as well as those with both echogenic liver and elevated aminotransferase activity at end of the study were considered as still having evidence of NAFLD. The time course of the changes in liver function is shown in Figures 1 (ALT, AST) and 2 (G-GT, AP).

Table 5. Multivariate analysis of predictors for the response to treatment that were found to be statistically significant in univariate models

	Independent variables	F value	p value
Dependent variable: absence of biochemical plus ultrasonographic NAFLD indices at the end of the study	Percentage change during treatment:		
	hsCRP	142.368	0.000
	Waist circumference	20.017	0.000
	Body weight	17.967	0.000
	TGs	9.457	0.003
	LDL-C	6.778	0.012
	Fasting glucose	5.645	0.021
	SBP	5.216	0.026
	TC	4.722	0.034
	SUA	3.555	0.064
	HDL-C	0.483	0.490
	DBP	0.693	0.408
	Absolute values:		
	Gender	1.669	0.201
	Age	0.070	0.792

Abbreviations as in Table 2

Predictors of response to treatment

No baseline features (clinical or laboratory) able to predict treatment outcome were found. Multivariate analysis showed that independent predictors of the response to treatment were the reductions in hsCRP (the most significant predictor as shown by the *F* value), waist circumference, body weight (BW), TGs, LDL-C, fasting glucose, systolic BP and total cholesterol (Table 5). For body weight in particular, a threshold of 4.2% reduction was predictive of a response to treatment. On the other hand, age, gender and changes in SUA levels, diastolic BP and HDL-C concentration did not independently predict treatment outcome (Table 5). As far as drug treatment (other than hypolipidaemic medication) is concerned, orlistat appeared to be associated with the most beneficial effect followed by metformin and ACEIs (Table 6).

Safety

Four patients discontinued treatment because of adverse effects. One patient from the atorvastatin group and two from the combination group discontinued treatment because of myalgia without elevated serum creatine kinase activity. One patient in the atorvastatin group developed an ALT activity > 3-fold the ULN.

Discussion

Currently, there is no established management of NAFLD. A recent review pointed out that 'we urgently need carefully controlled, randomised studies

Table 6. Multivariate analysis of drug treatment (other than hypolipidaemic medications) related to the response to treatment in the whole study population

Dependent variable: absence of biochemical plus ultrasonographic NAFLD indices at the end of the study	Independent variables:	F value	p value
	Orlistat	15.641	0.000
	Metformin	7.984	0.006
	ACEIs	7.435	0.008

ACEIs = angiotensin converting enzyme inhibitors

and specific programmes to simultaneously tackle all components of metabolic syndrome'¹⁰.

Our study provides evidence that target-driven intervention aimed at multiple risk factors in non-diabetic subjects with the MetS is safe and substantially reduces biochemical and ultrasonographic indices of NAFLD. This could be related to simultaneous interventions (e.g. hypolipidaemic treatment, weight loss, lowered BP and improved glucose metabolism, Tables 5 and 6). The improvement in liver function tests in these patients seems to occur within 9–12 months after the initiation of the multi-targeted treatment (Figures 1 and 2).

Insulin resistance is probably the key abnormality in NAFLD and MetS¹⁰. Insulin resistance increases adipose tissue lipolysis, resulting in increased efflux of free fatty acids (FFA) which are then delivered to the liver¹¹. The FFA are in turn esterified into TGs and incorporated into very low-density lipoprotein (VLDL). Excess TG-rich VLDL secretion by the liver leads to hypertriglyceridaemia. In addition, insulin resistance inhibits peripheral lipoprotein lipase activity, reduces VLDL clearance and further increases serum

TG levels¹¹. When FFA delivery to the liver or hepatic de novo synthesis exceeds TG export or oxidation, NAFLD may occur^{11,12}. Thus, the MetS, affecting one in four adults and one in two over the age of 60 years⁷ is often associated with this potentially progressive liver disease¹⁰. Insulin resistance is closely associated with visceral adiposity⁵ and reduction of waist circumference was the second most significant predictor of response to treatment in our study (Table 5).

In this study the mean weight reduction was more than 10% of baseline BW. It is widely accepted that a weight reduction of 5% is necessary to achieve meaningful clinical benefits. Weight loss after restricted diet and exercise¹³ or surgery in severely obese subjects¹⁴ provides major improvement or resolution of obesity and MetS-associated abnormal liver histological features. In fact, even modest weight loss alone is associated with normalisation of transaminases¹⁵. Indeed, a threshold of 4.2% reduction of initial body weight was predictive of a response to treatment in our cohort. Orlistat, a gastrointestinal lipase inhibitor, contributed to weight reduction in conjunction with hypocaloric diet in obese subjects (> 30 kg/m²)¹⁶. This may have had several beneficial effects on glucose metabolism, systolic BP, TC, LDL-C and insulin sensitivity¹⁷. A recent study showed that orlistat normalised the ultrasonographic evidence of NAFLD in overweight patients with hyperlipidaemia¹⁸. In addition, some preliminary evidence for histological improvement of non-alcoholic steatohepatitis exists for orlistat¹⁹. Indeed, multivariate analysis showed that weight loss (Table 5) and the use of orlistat (Table 6) were independent predictors of response to treatment.

There was a significant improvement in glucose status (Table 4) in our patients probably due to improved insulin sensitivity related to the low calorie–low fat diet, exercise and orlistat. Moreover, we treated MetS patients with IFG at baseline with metformin, which has been reported to reduce aminotransferase activity and improve histological findings in subjects with NAFLD^{20,21}. Thiazolidinediones (insulin-sensitising agents) have also been shown to be beneficial²². Multivariate analysis showed that the decrease of fasting glucose (Table 5) and the use of metformin (Table 6) were independently associated with treatment outcome.

Hypertensive patients have a significantly higher prevalence of NAFLD and hypertension is independently associated with high ALT activity²³. Our anti-hypertensive treatment was mainly based on inhibitors of the renin–angiotensin system (mainly ACEIs). In a recent study²⁴ the administration of an ARB, losartan, in patients ($n = 7$) with NAFLD and hypertension improved hepatic necroinflammation (in five patients)

and reduced hepatic fibrosis (in four patients) without any change in insulin sensitivity. Multivariate analysis showed that the reduction of systolic but not diastolic BP (Table 5) and the use of ACEIs (Table 6) were independently associated with the treatment response. The reasons for the observed difference between systolic and diastolic BP are not well understood and should be further studied. Unfortunately, the number of patients receiving an ARB was too small (compared with those using an ACEI) to be included in the analysis of who responded to treatment and who did not.

Reduction of levels of LDL-C, total cholesterol and triglycerides but not the rise in HDL-C were independent predictors of treatment outcome (Table 5). Atorvastatin was effective in reducing biochemical and ultrasonographic evidence of NAFLD in our patients. This is in accordance with previous reports in humans^{18,25} and experimental models^{26,27}. This could be related to the decrease in hepatic TG secretion without an increase in hepatic TG concentration²⁶, the reduction in hepatic FFA levels²⁶ and the reduction in cholesteryl ester availability derived from newly synthesised cholesterol that limits the secretion of VLDL²⁷.

Fibrate-induced peroxisome proliferator-activated receptor activation can stimulate the oxidative metabolism of FFA in the liver, thus reducing the quantity of FFA available for VLDL synthesis and secretion and the potential for lipid deposition in the liver²⁸. Moreover, fibrates reduce AP activity in hypertriglyceridaemic/dyslipidaemic subjects²⁹.

All hypolipidaemic treatments reduced SUA levels (Table 4). Atorvastatin³⁰ and fenofibrate³¹ can reduce SUA levels. Elevated SUA levels have been considered as a risk factor for the development of insulin resistance³² and a feature of the MetS³³. Furthermore, after multiple logistic regression analysis only SUA levels (and fasting insulin) were independent predictors of NAFLD in another study³⁴. However, in the present study the predictive power of SUA level reduction did not reach statistical significance in the multivariate analysis ($p = 0.064$, Table 5).

Multi-targeted treatment of MetS significantly reduced hsCRP levels in all three treatment groups. In fact, the decrease of hsCRP concentration was the most powerful predictive parameter of treatment outcome (Table 5). There seems to be a relationship between insulin resistance and markers of inflammation such as hsCRP, plasminogen activator inhibitor type 1, fibrinogen and cellular adhesion molecules in insulin-resistant conditions³⁵. Insulin resistance and hsCRP were independent risk factors for NAFLD in non-obese men, free of DM and hypertension³⁶. In addition, hepatic inflammation secondary to liver steatosis may be a potential contributor to the low-

grade inflammation associated with the MetS³⁷. There is growing evidence that hsCRP has direct pro-inflammatory effects and is an independent risk factor for coronary heart disease³⁸. We recently showed that the prevalence of atherosclerotic disease is high among subjects with MetS³⁹. Moreover, multi-targeted treatment of MetS resulted in a significant reduction in hsCRP levels, which correlated with the fall in the estimated 10-year risk for coronary heart disease⁷.

Atorvastatin and combination treatment were significantly more effective than fenofibrate alone in reducing the surrogate indices of NAFLD in our population. This can be attributed to differences in drug action^{26,27,40,41}. For example, potential differences in hsCRP reduction (−66% in the atorvastatin group vs. −43% in the fenofibrate group, $p = \text{NS}$) might be relevant since this marker of inflammation can activate complement, recruit monocytes and upregulate adhesion molecules and chemo-attractant chemokines³⁸. Furthermore, atorvastatin has a potent antioxidant effect⁴², thus influencing a factor involved in the pathogenesis of NAFLD⁴³. From the independent predictors of treatment outcome (Table 5), levels of total and LDL cholesterol were reduced significantly more in atorvastatin and combination groups compared with the fenofibrate group ($p < 0.05$, Table 4). On the other hand, no significant differences were observed among the three groups for changes in the other predictors. Therefore, this greater reduction of total and LDL cholesterol concentration in the atorvastatin and combination groups may explain the superiority of these treatment modalities over fenofibrate monotherapy.

The prevalence of NAFLD features in MetS subjects in our study was 62% (186 out of 300 subjects included in the original study). This is not unusually high since other studies have shown that an estimated 80% of MetS individuals have features of NAFLD^{44,45}.

There was no serious liver toxicity in our patients despite abnormal liver function tests at baseline. This finding is in keeping with previous studies showing that statins and fibrates are safe hypolipidaemic drugs even in patients with elevated baseline aminotransferase activity^{46,47}. As an added precaution we excluded patients with markedly abnormal liver function tests ($\times 3$ ULN) and there was regular monitoring during the study. When managing the MetS there is a need to consider that without hypolipidaemic treatment these patients have a high risk of a vascular event⁵.

Study limitations

There was no practical guidance to estimate sample size. Thus, we could not estimate the effect of the

treatment options we selected on liver echogenicity and aminotransferase activity.

A liver biopsy provides definitive diagnosis, evaluates the degree of inflammation or fibrosis and establishes a baseline for monitoring NAFLD progression⁴⁸. Potentially progressive NAFLD may exist without raised aminotransferase activity and this marker cannot be used to predict the presence of NAFLD in patients with MetS⁴⁸. Moreover, liver ultrasonography does not correlate with the degree of NAFLD severity¹⁰. However, ethical restrictions and the reluctance of patients with nearly normal liver function limit the use of liver biopsy. Moreover, several prospective cohort studies evaluating NAFLD did not include liver biopsies^{49–55}. Our patients were selected so as to have both biochemical and ultrasonographic evidence of NAFLD and other possible causes of liver disease were excluded. Thus, NAFLD was the most probable diagnosis. Additionally, both biochemical and ultrasonographic indices had to be normalised before concluding that a patient no longer had evidence of NAFLD. Whether these criteria correlate with the degree of histologic improvement and predict a better liver outcome in MetS patients is not known and should be further studied. Our results justify the need for a randomised study that includes liver histology.

A control group, consisting of MetS patients with evidence of NAFLD who did not receive specific therapy, would allow estimation of the percentage of patients in whom the indices of NAFLD spontaneously improve. However, withholding treatment in such high-risk patients is ethically challenging^{56,57}.

Conclusions

Target-driven intervention aimed at multiple risk factors in non-diabetic patients with MetS is safe and substantially offsets MetS component features and both biochemical and ultrasonographic evidence of NAFLD. These effects could be related to hypolipidaemic drug treatment and the administration of orlistat, metformin and ACEIs as well as the reduction in hsCRP, waist circumference, BW, TGs, LDL-C, fasting glucose, systolic BP and total cholesterol. The atorvastatin–fenofibrate combination and atorvastatin monotherapy were more effective than fenofibrate alone in reducing both biochemical and ultrasonographic evidence of NAFLD despite the fact that all the other potentially beneficial treatments were equally administered in the fenofibrate group. NAFLD and atherosclerotic disease may progress in parallel within the MetS⁶ or type diabetes⁵⁷. Thus, the treatment options described here may benefit both the heart and liver.

Acknowledgements

Declaration of interest: This study was conducted independently; no company or institution supported it financially.

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Paper CMRO-3408_2, *Accepted for publication*: 21 March 2006

Published Online: 04 April 2006

doi:10.1185/030079906X104696