Safety and impact on cardiovascular events of long-term multifactorial treatment in patients with metabolic syndrome and abnormal liver function tests: a *post hoc* analysis of the randomised ATTEMPT study

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Abstract

Introduction: Non-alcoholic fatty liver disease (NAFLD), a hepatic manifestation of metabolic syndrome (MetS), is common and accounts for 80% of cases of elevated liver function tests (LFTs). We assessed the long-term effects of multifactorial intervention on LFTs and their association with cardiovascular disease (CVD) events in patients with MetS without diabetes mellitus or CVD.
Material and methods: This prospective, randomized, open label study included 1,123 patients (aged 45-65 years). Patients received intensive lifestyle intervention and pharmacotherapy: atorvastatin in all patients (low density lipoprotein cholesterol [LDL-C] targets of < 100 mg/dl [group A] or < 130 mg/dl [group B]), inhibitors of the renin-angiotensin-aldosterone axis for hypertension, metformin for dysglycaemia and orlistat for obesity.
Results: Among participants, 326 had modestly elevated LFTs and ultrasonographic (US) evidence of NAFLD (165 patients in group A2 and 161 patients in group B2). The NAFLD resolved during the 42-month treatment period in 86% of patients in group A2 and in 74% of patients in group B2 ($p < 0.001$). In both groups nearly 90% of patients attained lipid goals. Mean LDL-C and TG levels were higher in group B2 than in group A2 ($p < 0.001$). There were no CVD events in group A2 whereas 5 non-fatal events occurred in group B2 (log-rank-$p = 0.024$). There were no major side-effects.
Conclusions: Attaining multiple treatment targets is safe and beneficial in primary prevention patients with MetS and NAFLD. Lipid levels and LFTs normalized, US findings associated with NAFLD resolved and no CVD events occurred in patients with LDL-C levels < 100 mg/dl (group A2). Resolution of NAFLD might have contributed to the prevention of CVD events.

Key words: non-alcoholic fatty liver disease, metabolic syndrome, multifactorial treatment, atorvastatin, cardiovascular disease.

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is a term used for a wide spectrum of hepatic pathology ranging from simple steatosis to non-alcoholic steatohepatitis (NASH) and cirrhosis in individuals who do not consume large amounts of alcohol [1]. There is growing interest in NAFLD because it accounts for approximately 80% of cases of elevated liver function tests (LFTs) in the American population and it affects approximately 20-25% of American adults [2-4]. Reports from Japan and Italy suggest a similar prevalence [5, 6]. In simple steatosis, present in up to 30% of the general population and in 75% of obese patients, triglycerides (TGs) accumulate in hepatocytes, occupying more than 5% of their volume. Simple steatosis has been reported to be associated with increased body mass index (BMI), abnormal glucose tolerance, insulin resistance and other components of the metabolic syndrome (MetS) [1, 7]. Accordingly, NAFLD is considered by some as the hepatic manifestation of MetS [8]. In turn, MetS confers an increased risk for cardiovascular disease (CVD) morbidity and mortality [9, 10] and all-cause mortality [9], even in the absence of CVD and/or diabetes mellitus (DM) [10, 11]. Patients with NAFLD have 69% higher all-cause mortality rates than the general population; patients with NASH have an even higher rate [12]. These higher rates are mainly due to CVD events and to a lesser extent due to liver disease-associated mortality [12]. In patients with both NAFLD and MetS the CVD risk appears to be even higher [8].

We recently showed that statin treatment, on top of optimal secondary prevention therapy, is safe and can improve LFTs and reduce CVD morbidity in patients with coronary heart disease with modestly abnormal LFTs, possibly due to NAFLD [13]. However, there are no data on the effect of multifactorial treatment, including a statin, on NAFLD and the related CVD events in primary prevention patients with MetS. This *post hoc* analysis of the Assessing The Treatment Effect in Metabolic Syndrome Without Perceptible diabeTes (ATTEMPT) study [14] was undertaken to address this issue.

Material and methods

The study protocol was described in detail in the original paper [14].

Study population

This is a prospective, randomised, target-based, intention-to-treat, open label study on a selected sample from throughout Greece. All participants had to meet the American Heart Association diagnostic criteria [15] for MetS and attended the participating outpatient clinics, considered to specialise in at least 1 MetS component. Specifically, at least 3 of the following criteria should be present: TG levels > 150 mg/dl (1.7 mmol/l); high density lipoprotein cholesterol (HDL-C) levels < 40 mg/dl (1.0 mmol/l) for men or < 50 mg/dl (1.3 mmol/l) for women; blood pressure (BP) > 130/85 mmHg or treatment for the latter 3 conditions; waist circumference (WC) > 102 cm in men or > 88 cm in women; and fasting plasma glucose (FPG) > 100 mg/dl (5.6 mmol/l) [15].

Exclusion criteria in our study were as follows: TG levels > 500 mg/dl (5.6 mmol/l), pregnancy or lactation, hormone replacement therapy, active hepatic disease (known hepatitis or unexplained persistent transaminases elevation > 100 IU/l), secondary or resistant hypertension, advanced chronic kidney disease (CKD) (estimated glomerular filtration rate < 30 ml/min/1.73 m^2) and the presence of CVD and/or DM (FPG $\geq$ 126 mg/dl (7 mmol/l) and/or glycosylated haemoglobin (HbA$_{1c}$) > 6%]. The CVD was excluded on the basis of personal history, physical examination and if needed an exercise stress test. Among screened patients, 1,320 agreed to be followed for a longer period (3.5 years) and 1,123 had the appropriate age range (45-65 years old) for CVD risk score estimation and were enrolled in the study and were randomised to the 2 treatment groups using block (of 4) randomization. Randomization was performed by VGA and the rest of the investigators were blinded to treatment.

Study protocol

The protocol of the ATTEMPT study included multifactorial targeting of all CVD risk factors, including components of the MetS, in all patients during a 42-month period.

Lifestyle advice: All subjects received lifestyle advice. This included exercise (walking for at least 30 min 5 days a week (150 min) or equivalent exercise) and hypolipidaemic (National Cholesterol Education Program Adult Treatment Panel III) hypocaloric diet [15]. At baseline there was an interview with a qualified dietician. Dietary preferences were recorded and a specific 7-day diet sheet was handed to the patient at every visit. Daily calorie intake was set at 500 calories below the needs of the patient according to physical activity.

Treatment of hypertension: Hypertension was treated in a stepwise manner with 5 mg/day (titrated up to 40 mg/day) of quinapril. If this was not enough to reach the treatment target, amlodipine (titrated from 5 to 10 mg/day), and then low-dose hydrochlorothiazide (12.5 mg/day) were added. If one more antihypertensive drug was required, then central acting antihypertensives, low

dose selective β-blockers, and finally any other drug were added to reach the treatment target. The intention was to avoid any metabolic side-effects of antihypertensive drug treatment.

Treatment of obesity: In patients with obesity (BMI > 30 kg/m^2) orlistat (up to 360 mg/day) was prescribed throughout the study period.

Treatment of impaired fasting glucose (IFG): The IFG was treated with metformin in patients with FPG > 110 mg/dl (6.1 mmol/l). The starting dose was 850 mg/day and was increased to 1,700 mg/day if FPG levels remained > 110 mg/dl (6.1 mmol/l).

Treatment of dyslipidaemia: Atorvastatin was administered to all subjects; these were randomly allocated to 2 treatment groups. Group A: LDL-C target < 100 mg/dl (n = 566) and group B: LDL-C target of < 130 mg/dl (n = 557). The atorvastatin dose was titrated from 10 to 80 mg/day.

The study protocol was approved by the local and national ethics committees and written informed consent was obtained from each participant before enrolment. The trial was registered 6 years ago in the official government of USA ClinicalTrials.gov (Trial registration number: ID: NCT00416741).

Investigated parameters

Data collected from the study subjects included a detailed personal medical history, measurements of the components of the MetS (blood lipids, BP, FPG and WC), as well as other CVD risk factors (sex, age, BMI, smoking status and family history of premature CVD). Blood samples were collected after a 12 h fast (water allowed) between 8 and 10 am, in a sitting position. Total cholesterol, HDL-C and TG levels were determined. The LDL-C was calculated by the Friedewald formula (LDL-C [mg/dl] = TC [mg/dl] − (TG [mg/dl]/5 + HDL-C [mg/dl])). Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transpeptidase (γ-GT), alkaline phosphatase (AP), creatinine (SCr), uric acid (SUA) and creatine kinase (CK) were also assessed. Estimated glomerular filtration rate (eGFR ml/min/1.73 m^2) was measured using the Modification of Diet in Renal Disease equation: eGFR = 175 × (SCr)−1.154 × (age)−0.203 × (0.742 if female) [http://www.nkdep.nih.gov/professionals/ gfr_calculators/idms_con.htm]. Biochemical evaluation was carried out in various laboratories that fulfilled internal and external validity control criteria. All measurements in the same centre were performed by the same lab, using the same methods, and therefore results are comparable. In patients with raised LFTs, abdominal ultrasound (US) was performed, and when US findings were suggestive of NAFLD, the patient was characterized as having NAFLD.

Endpoints

The primary endpoint of the original study was the reduction in the 10-year eCVD risk at 6 months compared with baseline (of the interventional part of the study, and not the baseline of the epidemiological part of the study) in the total study population (n = 1,123). The secondary endpoints were the eCVD risk reduction during the next 3 years, the proportion of patients having MetS at the 42nd month of treatment and the number of CVD events (unstable angina (UA), myocardial infarction (MI), percutaneous coronary intervention (PCI), coronary artery bypass grafting (CABG), stroke, transient ischaemic attack (TIA) and CVD death) in all participants. In the present *post hoc* analysis the primary endpoint was the effect of multifactorial treatment on the prevalence of NAFLD in patients with MetS and NAFLD in both treatment groups (n = 326) and its relation with actual CVD events during the 42-month follow-up. The secondary endpoint was the relation of NAFLD with chronic kidney disease (CKD).

Safety

A clinical and laboratory (ALT, AST, CK, SCr) investigation was performed every 6 months at programmed study visits (there were 10-12 such visits).

Statistical analysis

Intention-to-treat analysis of all patients randomized to the 2 treatment groups was performed. Continuous variables are presented as mean ± standard deviation (SD) since all parameters were normally distributed according to the Kolmogorov-Smirnov test. Qualitative variables are presented as relative frequencies. Clinical and laboratory findings between groups were compared by ANOVA. Unpaired Student's *t*-tests were used for comparisons of parametric data and χ^2 tests for comparisons of categorical data between the 2 groups at specific time points. The log-rank test was used to compare CVD event rates in the 2 treatment groups. A two-sided p < 0.05 was considered significant. All analyses were carried out using the SPSS 19.00 software package (SPSS, Inc., Chicago, IL).

Results

At baseline, 326 patients had modestly elevated LFTs and US evidence of NAFLD (165 patients in group A2 and 161 patients in group B2).

Effect of treatment on LFTs in patients with US evidence of NAFLD

There was a substantial reduction in all liver enzyme activities in patients with raised LFTs at

baseline and US evidence of NAFLD. In group A2 ALT was reduced by 48% (from 60 ±11 IU/l to 31 ±5 IU/l; $p < 0.001$), AST by 47% (from 51 ±10 IU/l to 27 ±4 IU/l; $p < 0.001$), γ-GT by 60% (from 91 ±15 IU/l to 36 ±6 IU/l; $p < 0.001$) and AP by 43% (from 171 ±28 IU/l to 85 ±9 IU/l; $p < 0.001$) during the 42-month follow-up (Table I and Figure 1, panel 1). In group B2 ALT was reduced by 42% (from 61 ±12 IU/l to 35 ±6 IU/l; $p < 0.001$), AST by 40% (from 52 ±11 IU/l to 31 ±6 IU/l; $p < 0.001$), γ-GT by 52% (from 93 ±14 IU/l to 44 ±6 IU/l; $p < 0.001$) and AP by 40% (from 169 ±32 IU/l to 101 ±15 IU/l; $p < 0.001$) during the 42-month follow-up (Table I and Figure 1, panel 2). Reductions in ALT, AST, γ-GT and AP in group A2

were larger than in group B2 ($p < 0.05$ for all 4 comparisons).

At the end of the study, 86% ($n = 142$) of the patients in group A2 had normal LFTs and no US evidence of NAFLD, 8% ($n = 13$) had normal LFTs but US evidence of NAFLD and 6% ($n = 10$) had both elevated LFTs and US evidence of NAFLD. In group B2, at the end of the study 74% of the patients ($n = 119$) had normal LFTs and no US evidence of NAFLD, 15% ($n = 24$) had normal LFTs but some US evidence of NAFLD and 11% ($n = 18$) had both elevated LFTs and US evidence of NAFLD. NAFLD resolution rate was higher in group A2 than in group B2 (86 vs. 74%; $p = 0.0012$).

Table I. Characteristics of patients with MetS and NAFLD according to treatment allocation (group A2, LDL-C target < 100 mg/dl; group B2, LDL-C target < 130 mg/dl)

Parameter	Patients with abnormal LFTs at baseline ($n = 326$)					
	Group A2 ($n = 165$)			Group B2 ($n = 161$)		
	Baseline	42 months	Value of p	Baseline	42 months	Value of p
Age [years]	58 ±7	–	–	57 ±9	–	–
Family history of CVD [%]	34	–	–	36	–	–
Smoking [%]	33	25	< 0.001	32	25	< 0.001
Body mass index [kg/m^2]	33 ±4	29 ±4	< 0.001	32 ±5	29 ±3	< 0.001
Weight [kg]	89 ±14	82 ±11	< 0.001	89 ±13	83 ±12	< 0.001
Systolic blood pressure [mmHg]	145 ±13	125 ±9	< 0.001	144 ±11	124 ±10	< 0.001
Diastolic blood pressure [mmHg]	91 ±9	80 ±4	< 0.001	90 ±10	79 ±5	< 0.001
HDL-C [mg/dl]	45 ±11	52 ±7	< 0.001	46 ±10	51 ±8	< 0.001
Triglycerides [mg/dl]	220 ±47	123 ±20	< 0.001	218 ±50	142 ±34*	< 0.001
Waist circumference [cm]	105 ±9	98 ±8	< 0.001	104 ±11	99 ±7	< 0.001
Blood glucose [mg/dl]	106 ±12	98 ±9	< 0.001	107 ±13	97 ±8	< 0.001
Total cholesterol [mg/dl]	255 ±44	165 ±24	< 0.001	256 ±41	186 ±26*	< 0.001
LDL-C [mg/dl]	168 ±38	92 ±12*	< 0.001	171 ±36	110 ±20*	< 0.001
Metabolic syndrome [%]	100	15	< 0.001	100	16	< 0.001
Abdominal obesity [%]	90	50	< 0.001	91	52	< 0.001
Hypertension [%]	85	11	< 0.001	84	10	< 0.001
High TG levels [%]	79	10	< 0.001	80	12	< 0.001
Impaired fasting glucose [%]	72	9	< 0.001	71	8	< 0.001
Low HDL-C [%]	61	8	< 0.001	60	7	< 0.001
ALT [IU/l])	60 ±11	31 ±5	< 0.001	61 ±12	35 ±6*	< 0.001
AST [IU/l]	51 ±10	27 ±4	< 0.001	52 ±11	31 ±6*	< 0.001
γ-GT [IU/l]	91 ±10	36 ±5	< 0.001	93 ±11	44 ±6*	< 0.001
Alkaline phosphatase [IU/l]	171 ±28	86 ±9	< 0.001	169 ±32	101 ±15*	< 0.001
CVD events	Expected events: 8-11 actual CVD events: 0			Expected events: 10-13 actual CVD events: 5 ($p = 0.024$)		

*$p < 0.001$ vs. group A2, MetS – metabolic syndrome, NAFLD – non-alcoholic fatty liver disease, LFTs – liver function tests, CVD – cardiovascular disease, LDL-C – low density lipoprotein cholesterol, HDL-C – high density lipoprotein cholesterol, TG – triglycerides, ALT – alanine aminotransferase, AST – aspartate aminotransferase

LTs in group A2

LTs in group B2

Event free survival of A2 and B2

log-rank *p* = 0.024
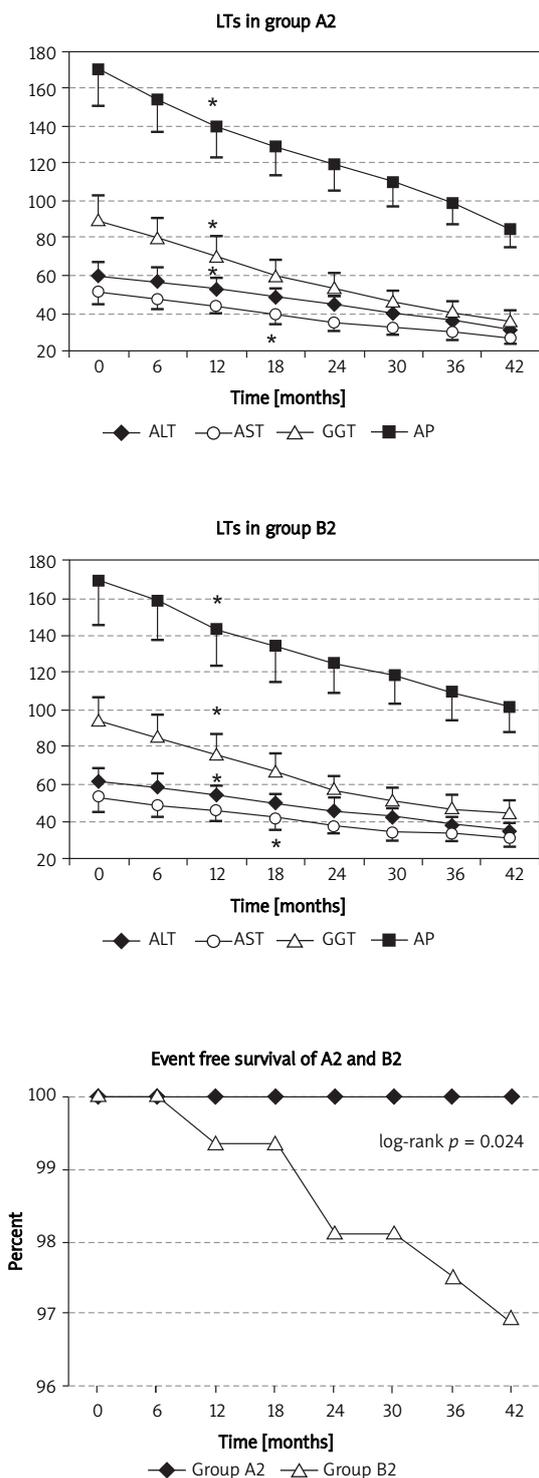
Figure 1. Time course of changes in alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transpeptidase (γ-GT), and alkaline phosphatase (AP) levels (panel 1 for group A2 (*n* = 165) and panel 2 for group B2 (*n* = 161)) and event-free survival during the study in groups A2 and B2 (panel 3)

*p < 0.05 vs. baseline, group A2 – patients with an LDL-C target < 100 mg/dl, group B2 – patients with an LDL-C target < 130 mg/dl

Effects of treatment on other parameters in patients with NAFLD

Multifactorial treatment in patients with NAFLD

Atorvastatin was used in all patients with forced dose titration (10 mg/day to 80 mg/day). In patients with NAFLD, the mean dose of atorvastatin during the study was 34 mg/day and 24 mg/day in group A2 and B2, respectively. The adherence to atorvastatin during the study was high (92%). The LDL-C and TGs were significantly higher (*p* < 0.05) in group B2 than in group A2 during the study (Table I). Hypertension was treated with quinapril (mean dose 20 mg/day in both groups; *n* = 229, 70% of patients) and if needed with amlodipine (mean dose 8 mg/day in both groups; *n* = 131, 40% of patients), and then, if needed, with hydrochlorothiazide (mean dose 12.5 mg/day in both groups; *n* = 83, 25% of patients). About 28% of patients were on angiotensin receptor blockers (ARBs), 12% on centrally acting antihypertensive drugs, 10% on β-blockers and 17% on various other antihypertensive agents. In patients with evidence of NAFLD and a BMI > 30 kg/m^2, orlistat was used in a mean dose of 290 mg/day (*n* = 169). Metformin was used in a mean dose of 950 mg/day in 152 patients with IFG.

The use of drugs comprising the multifactorial intervention and the doses administered (with the exception of atorvastatin) did not differ between treatment groups (Table I). Only the atorvastatin doses used and the lipid values achieved differed significantly between the 2 treatment groups.

Changes in measured parameters in patients with NAFLD

MetS components: The most common MetS component was central obesity, followed by hypertension and high TG and FPG levels and lastly HDL-C. The most prevalent phenotype (19%) was that with elevated BP (or on antihypertensive treatment), increased WC, high TGs and increased FPG. Baseline LDL-C levels were 170 mg/dl. None of the measured parameters differed significantly between groups A2 and B2 at baseline. At the end of the study, only 10% of the patients with NAFLD still had MetS (persistent cases), but with fewer components.

Lipid levels: At the 42nd treatment month LDL-C was substantially reduced in both groups (92 ±12 mg/dl in group A2 vs. 110 ±20 mg/dl in group B2, *p* < 0.001) (Table I). Approximately 90% of the study population achieved the allocated LDL-C target (< 100 mg/dl in group A2 and < 130 mg/dl in group B2; Table I). Patients in group A2 had lower TG levels than group B2 (123 ±20 mg/dl vs. 142 ±34 mg/dl, *p* < 0.001). The HDL-C levels showed a comparable increase in both groups.

Renal function: A substantial proportion of patients with NAFLD (137/326, 42%) had stage 3 CKD (eGFR 30-59 ml/min/1.72 m^2). This proportion was higher than in patients without NAFLD (212/797, 26%; $p < 0.001$). In patients with NAFLD and stage 3 CKD, eGFR was increased by 13% vs. baseline ($p < 0.001$).

Other CVD risk factors: The BMI, FPG levels and the prevalence of hypertension were significantly reduced during the 42 months of treatment (Table I). Smaller but significant improvements were observed in WC.

New onset diabetes (NOD): The incidence of NOD during the 42-month follow-up was very low, 0.83-1.00/100 patient-years in patients with MetS and MetS with impaired fasting glucose, respectively.

High sensitivity C-reactive protein (hsCRP) levels: The hsCRP levels declined in group A2 more than in group B2 (from 4.0 mg/l at baseline to 0.7 mg/l at 42 months in group A2 ($p < 0.001$) and from 4.1 mg/l to 1.4 mg/l over the same time period in group B2 ($p < 0.001$); $p < 0.05$ for the comparison between the 2 groups during the entire study).

The CVD events in NAFLD patients

During the first 6 months there were no CVD events among the 326 participants with NAFLD. Between the 6th and the 42nd month there was no CVD event in group A2 (Table I and Figure 1, panel 3) whereas 5 non-fatal CVD events (3 non-fatal MIs and 2 PCIs) occurred in group B2 ($p = 0.0024$, Table I).

Effect of treatment on LFTs in patients without US evidence of NAFLD

At baseline 42 patients, apart from the 326 with NAFLD, had elevated LFTs without US confirmation of NAFLD. These had ALT and AST values similar to those of the 326 NAFLD patients but lower values of γ-GT and AP (65 ±14 IU/l and 132 ±13 IU/l vs. 92 ±12 and 170 ±25 IU/l, respectively; $p < 0.05$). The reduction in both ALT and AST during the study was lower than that achieved in the 326 NAFLD patients (26 vs. 45% in ALT and 24 vs. 43% in AST values ($p < 0.05$ in comparison to the reduction of ALT and AST values in the 326 NAFLD patients)).

Discussion

We recently reported that statin treatment is safe and can improve LFTs and reduce CVD morbidity and mortality in secondary prevention patients with modestly abnormal LFTs that were attributable to NAFLD [13]. To our knowledge, the present study is the first prospective, randomized, intention-to-treat, controlled trial to assess the effects of multifactorial intervention on LFTs and related CVD events in primary prevention patients with NAFLD. The key findings are that this multifactorial intervention results in an improvement of LFTs and reduces the CVD event rate. These findings could be attributed to intensive atorvastatin treatment and lower lipid levels, because reductions in LFTs and CVD events in group A2 (with an LDL-C < 100 mg target) were superior to those in the less aggressive group B2 (with an LDL-C target < 130 mg/dl), while all other risk factors and interventions were similar in these 2 groups.

Visceral obesity, impaired glucose metabolism, hypertension and dyslipidaemia are prevalent in NAFLD, and this might play a role in the increased CVD risk in NAFLD patients [16]. However, it appears that NAFLD *per se* might be independently related to increased CVD risk [17-19]. In NAFLD, insulin resistance is related to elevated levels of markers of inflammation, such as CRP and fibrinogen [17]. Moreover, in obesity/NAFLD visceral adipocytes are a major source of the increase in circulating proinflammatory, prothrombotic and proatherogenic factors, collectively termed adipokines, which include tumour necrosis factor-α, leptin, plasminogen activator inhibitor-1, interleukin-6 and angiotensinogen, whereas levels of adiponectin, which has antidiabetic, anti-atherogenic and anti-inflammatory properties, are substantially reduced [17, 18]. Thus, increased pro-atherogenic factors and decreased plasma levels of adiponectin may represent another possible mechanism linking NAFLD and CVD [17-19]. Most of these factors appear to normalize during statin treatment [20].

Given the multifactorial pathogenesis of NAFLD, numerous treatments for various contributory risk factors have been proposed in the past, but there is no single established intervention to be recommended for all cases irrespective of the primary NAFLD risk factor [21]. Weight loss and increased physical activity constitute the cornerstone of NAFLD treatment [21]; however, the compliance of patients to long-term lifestyle modifications is low and therefore pharmacotherapy is frequently necessary [22, 23]. Even modest weight loss (7-10% of body weight) in overweight patients with NAFLD has been shown to improve LFTs [24]. In addition, improvement or resolution of obesity and MetS has been associated with major improvements in lobular steatosis as well as in liver necroinflammation and fibrosis [24, 25]. In ATTEMPT, intensive lifestyle measures resulted in a 7.3% weight loss in NAFLD patients (Table I). The use of orlistat is a safe adjunct to intensive lifestyle measures for weight loss [26]. Orlistat, either monotherapy or in combination therapy, also appears to be beneficial in NAFLD [26-30]. In the present study, significant reductions were observed in body weight, BMI and WC, and there is evidence that weight loss induced by orlistat might reverse

fatty infiltration of the liver and improve insulin resistance and even hepatic fibrosis in obese patients with NAFLD [31]. Thus, the use of orlistat in 51% of our patients might have contributed to the resolution of NAFLD.

It has also been shown that both metformin and dietary treatment alone ameliorate liver steatosis and metabolic abnormalities in patients with NAFLD [32]. However, metformin plus diet was more effective than dietary treatment alone in normalizing several metabolic parameters in these patients [32]. In contrast to thiazolidinediones, metformin has been shown to improve biochemical markers in NAFLD patients without inducing weight gain [33-35]. In addition, an improvement in body composition and fat distribution as well as a reduction in liver necroinflammation was observed in patients treated with metformin [22]. Thus, the use of metformin by 46% of our patients might have also played a role in the resolution of NAFLD.

Hypertension appears to be associated with NAFLD [36], and in the ATTEMPT study, hypertension was the most prevalent MetS component [14]. The renin-angiotensin-aldosterone system (RAAS) plays a role in increasing insulin resistance whereas RAAS suppression improves intracellular insulin signalling through activation of PPAR-γ receptors [37-39]. This effect attenuates hepatic inflammation and fibrogenesis, which translate into clinical and histological improvement in NAFLD [37-39]. Thus, the fact that 70% of our patients were on an ACE-I and 28% on an ARB might have contributed to the improvement in LFTs [36].

Nevertheless, all the above treatments, except for atorvastatin, were equally administered to both treatment groups. Therefore, we mainly attribute the greater reductions in LFT values, higher rates of NAFLD resolution and lower CVD incidence in group A2 compared with group B2 to more aggressive treatment with atorvastatin. The pleiotropic effects of statins, which also appear to be dose related, might have also played a role in the improved outcome of patients who received higher atorvastatin doses [16, 40].

We have previously shown that atorvastatin alone or in combination with fenofibrate normalizes LFTs and US findings in patients with NAFLD, free of DM and CVD at baseline [41]. These effects were independently related to statin treatment as well as to reductions in WC, body weight, systolic BP and serum hsCRP, TG, LDL-C, total cholesterol and FPG levels [41]. Other studies have also shown that statins are safe and effective in patients with elevated LFTs because of NAFLD [42, 43]. In a study with histopathological follow-up, NAFLD patients who were on statins exhibited significant reductions in liver steatosis as opposed to patients who were not taking statins [43]. Recently, we showed in patients with coronary heart disease that, besides the resolution of LFTs and improvement in US findings, statin treatment reduced CVD events more in patients with NAFLD than in those without NAFLD (68% vs. 39%, respectively; p = 0.007) [13, 44]. The present $post\ hoc$ analysis of the ATTEMPT study suggests, for the first time in primary prevention, that a higher atorvastatin dose (34 mg/day), which achieved lower LDL-C and TG levels, reduced LFTs values and CVD events more than a lower dose of atorvastatin (24 mg/day). This suggests that to achieve maximum benefit, more aggressive LDL-C targets should be pursued for dyslipidaemia associated with MetS-related NAFLD. In ATTEMPT, the differences in LDL-C and TG levels between groups A2 and B2 were 18 and 19 mg/dl, respectively (Table I), whereas there was no significant difference in HDL-C levels between the 2 treatment groups. There are no survival studies that included patients with MetS and NAFLD. However, our findings concur with the effects of atorvastatin on lipids and on CVD incidence in a $post\ hoc$ analysis of the Treating to New Targets (TNT) trial in patients with MetS [45]. In patients with CVD and MetS included in TNT, the mean on-treatment LDL-C concentrations at 3 months were 99 mg/dl with atorvastatin 10 mg/day and 72 mg/dl with atorvastatin 80 mg/day (a difference of 27 mg/dl; p < 0.001), TGs were 148 mg/dl and 176 mg/dl respectively (a difference of 28 mg/dl; p < 0.001) and there was no significant difference in HDL-C levels between the 2 treatment groups [45]. At a median follow-up of 4.9 years, major CVD events occurred in 13% of the patients receiving atorvastatin 10 mg/day and in 9.5% of the patients receiving atorvastatin 80 mg/day (29% reduction, p < 0.001) [45]. Similar findings were observed in MetS patients without DM [45]. A subsequent subgroup analysis of the Scandinavian Simvastatin Survival Study showed that patients with MetS benefited from simvastatin therapy by at least as much as those without MetS, despite the relatively high baseline (190 mg/dl) and on-study (120 mg/dl) values of LDL-C [46].

Another possible explanation for NAFLD resolution and additional CVD clinical benefit induced by atorvastatin is that liver TG increase is linked with decreased expression of PPAR-α, which is prevented by atorvastatin treatment [47]. The increase in PPAR-α expression caused by atorvastatin is associated with reduced liver TGs and plasma non-esterified fatty acid levels, because of increased hepatic fatty acid β-oxidation activity [47]. Moreover, atorvastatin can ameliorate the very low density lipoprotein (VLDL) apolipoprotein B overproduction state observed in a fructose-fed, insulin-resistant hamster model, and may also

improve hepatic insulin sensitivity [48]. In addition, MetS-induced abnormalities in plasma markers of inflammation and haemostasis and in monocyte secretory functions are ameliorated by atorvastatin [49]. Indeed, in ATTEMPT a substantial fall in hsCRP levels was recorded. Finally, atorvastatin substantially reduced SUA levels, which are also implicated in NAFLD-related CVD risk [50].

We also observed a significant association between NAFLD and the risk of CKD, 2 independent CVD risk factors [51], which share many important cardio-metabolic risk factors [52]. The prevalence of stage 3 CKD among NAFLD patients was higher than in those without NAFLD (42% vs. 26%; $p < 0.001$), suggesting a common pathophysiology [52, 53]. Inter-related risk factors, dyslipidaemia and proinflammatory/procoagulant mediators from the fatty/inflamed liver, might play a role in the natural history of obesity-related kidney and liver disease [52, 53]. The coexistence of these 2 risk factors appears to further increase the CVD risk [19, 51]. We showed in another *post hoc* analysis of ATTEMPT that multifactorial treatment improves renal function and lowers SUA levels in patients with stage 3 CKD [54]. This is also true for patients with NAFLD and CKD (a 13% increase in eGFR was observed) and might have acted in conjunction with NAFLD resolution to minimize CVD events in patients with MetS, NAFLD and CKD. The fact that the incidence of NOD during the 42-month follow-up was very low (0.83-1.00/100 patient-years) in patients with MetS and MetS with impaired fasting glucose, respectively, might have contributed to the low incidence of CVD in our patients [55].

Our findings suggest that atorvastatin therapy might prevent hepatocellular damage [56]. It was shown that the combination of atorvastatin with a standard diet yields greater improvements in NASH [56]. Thus, atorvastatin therapy not only decreases the levels of cholesterol and TGs, but might also induce a reduction of liver steatosis, inflammation and hepatocellular damage, which will translate to reduction of LFT values [56].

It is possible that most of the 42 patients who had elevated LFTs without US findings of NAFLD had indeed a "milder" form of NAFLD (with less fat deposition). These patients had significant but smaller reductions in ALT and AST than those with both LFT elevations and US findings of NAFLD (n = 326). One possible explanation is that multifactorial treatment significantly reduced the higher liver fat content in the latter patients, leading to a greater reduction of LFTs.

This study has several limitations. This is a *post hoc* analysis of the original survival study with MetS patients having NAFLD. We did not perform biopsies to diagnose NAFLD or its resolution after treatment.

Biopsy is the "gold standard" for diagnosis of NAFLD; hence we tried to strengthen our diagnostic criteria in the light of ethical restrictions associated with liver biopsies (especially if this would be required before and after treatment in a large number of patients). This is why we included both features, LFTs and US. The number of patients analysed is relatively small. Generalisability of study findings will be reinforced by prospective data of larger studies.

In conclusion, within a multifactorial treatment, it is safe to prescribe statins in primary CVD prevention patients with MetS and NAFLD with modestly elevated LFTs. The LFTs are gradually reduced and US findings improve during long-term statin treatment. Moreover, there is a substantial reduction in CVD events when lipid targets are attained. These findings support our earlier suggestion for statin use in NAFLD treatment [57]. Given the high prevalence of NAFLD and its role as a CVD risk factor, properly designed, prospective studies are needed to confirm these findings and allow the incorporation of statin treatment as a major component of NAFLD therapy.

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Thirty collaborators

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