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(Article begins on next page)

A Meta-Analysis of Randomized Trials for the Treatment of Nonalcoholic Fatty Liver Disease

Giovanni Musso,¹ Roberto Gambino,² Maurizio Cassader,² and Gianfranco Pagano²

Nonalcoholic fatty liver disease (NAFLD) encompasses a histological spectrum ranging from simple steatosis to nonalcoholic steatohepatitis (NASH). NAFLD carries a higher risk of cardio-metabolic and liver-related complications, the latter being confined to NASH and demanding specific treatment. We assessed the efficacy of proposed treatments for NAFLD/NASH by reviewing reports of randomized controlled trials (RCTs) on online databases and national and international meeting abstracts through January 2010. Primary outcome measure was histological improvement; secondary outcome was biochemical improvement; improvement in radiological steatosis was also evaluated. Two reviewers extracted articles using predefined quality indicators, independently and in duplicate. Main outcomes of randomized controlled trials (RCTs) were pooled using random-effects or fixed-effects models. Publication bias was assessed by funnel plots. Forty-nine RCTs (30 in NASH) were included: 23 RCTs (22 in NASH, 1 in NAFLD) had post-treatment histology. Most RCTs were small and did not exceed 1-year duration. Weight loss, thiazolidinediones (especially pioglitazone), and antioxidants were most extensively evaluated. Weight loss was safe and dose-dependently improved histological disease activity in NASH, but more than 50% of patients failed to achieve target weight loss. Thiazolidinediones improved steatosis and inflammation but yielded significant weight gain. RCTs with antioxidants yielded conflicting results and were heterogeneous with respect to type and dose of drug, duration, implementation of lifestyle intervention. Among the other agents, pentoxifylline, telmisartan and L-carnitine improved liver histology in at least 1 RCT in NASH; polyunsaturated fatty acid (PUFA) ameliorated biochemical and radiological markers of NAFLD. Other approaches yielded negative results. **Conclusion:** Well-designed RCTs of adequate size and duration, with histological endpoints, are needed to assess long-term safety and efficacy of proposed treatments on patient-oriented clinical outcomes. (HEPATOLOGY 2010;52:79-104)

Nonalcoholic fatty liver disease (NAFLD) affects 30% of the general Western adult population.¹ NAFLD ranges from simple steatosis to nonalcoholic steatohepatitis (NASH), characterized by steatosis plus necro-inflammation, which can only be differentiated by liver biopsy. NASH can progress

Abbreviations: BS, bariatric surgery; CI, confidence interval; FFA, free fatty acid; FLIRT, Fatty Liver Improvement with Rosiglitazone Therapy; HQ, high-quality; HOMA, Homeostasis Model Assessment; MRS, magnetic resonance spectroscopy; NAFLD, nonalcoholic fatty liver disease; NAS, nonalcoholic fatty liver disease activity score; NASH, nonalcoholic steatohepatitis; PPAR, peroxisome proliferators-activated receptor; PUFA, polyunsaturated fatty acid; RCT, randomized controlled trial; TZD, thiazolidinediones; UDCA, ursodeoxycholic acid.

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to cirrhosis and end-stage liver disease and is projected to be the leading cause of liver transplantation by 2020; furthermore, emerging evidence suggests that NAFLD overall is associated with an increased cardiometabolic risk.¹ We systematically reviewed the evidence in the treatment of NAFLD and NASH.

Patients and Methods

Data Sources and Searches

Databases searched through January 2010 were: MEDLINE, Ovid MEDLINE In-Process, Cochrane CENTRAL Register of Controlled Trials; Cochrane Database of Systematic Reviews, Excerpta Medica Database, Pubmed, clinicaltrials.gov, and American Association for the Study of Liver Diseases/American Gastroenterological Association/European Association for the Study of the Liver/Digestive Disease Week meeting abstracts, which were subjected to the same assessment as regular articles. We contacted ten authors to verify results and methodological quality of retrieved articles (see Acknowledgment).

Search terms were: NASH, NAFLD, nonalcoholic steatohepatitis, nonalcoholic fatty liver disease, fatty liver, liver fat, steatosis, AST, ALT, GGT, aminotransferase, liver enzymes, management, therapy, treatment, trial.

Study Selection

Inclusion criteria were English and non-English articles with participants aged older than 12 years, of any sex or ethnic origin with NAFLD/NASH, diagnosed on the basis of radiological/histological evidence of fatty liver.

Exclusion criteria were nonhuman studies, non-randomized trials, letters/case reports, studies enrolling fewer than 10 subjects, articles not reporting outcomes of interest or primary data (editorials, reviews), studies using inadequate case definitions or enrolling secondary steatosis (for example, drug-induced, total parenteral nutrition-induced steatosis).

Outcome Measures. Primary outcome assessed was histological response (number of patients with improvement in the degree of steatosis, inflammation, and fibrosis). When post-treatment histology was unavailable, biochemical response (alanine aminotransferase [ALT] responses, reported in all trials, was reported in the text; aspartate aminotransferase and gamma-glutamyl transpeptidase responses, less commonly reported, were described in online-only figures), and radiological response (improvement in steatosis by ultrasound, computed tomography, nuclear magnetic

resonance/spectroscopy) were evaluated. The effect on cardiometabolic risk profile and adverse events were also evaluated.

Data Extraction and Quality Assessment

Data were extracted independently and in duplicate by two authors (G.M., G.P.); discrepancies were resolved by mutual discussion. The agreement between the two reviewers for selection and validity assessment of trials was scored by kappa coefficient.

The quality of randomized controlled trials (RCTs) was assessed by the Cochrane Risk of Bias Tool, attributing 1 point to each item. Additionally we assessed if sample size was calculated *a priori* (total score range: 0-9; Table 1).² Meta-analyses following Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines were also included.³

Data Synthesis and Analysis

We used WinBUGS 1.4 (WinBUGS 1996-2003, Imperial College of Science & MRC, UK). The analysis was carried out according to the Cochrane Handbook of Systematic Reviews.² Dichotomous variables were presented as odds ratios with 95% confidence interval (CI), continuous variables as weighted mean differences with 95% CI. The fixed-effect model was used, with significance set at $P = 0.05$. Statistical heterogeneity was assessed using the I^2 statistic: with I^2 values of 50% or greater, we used a random-effects model and explored individual study characteristics and those of subgroups of the main body of evidence. Sensitivity analysis was performed by removing one study at a time and repeating the meta-analysis to assess whether any one study significantly affected pooled estimates. Additionally, we planned *a priori* subgroup analysis according to the following criteria: NASH versus NAFLD population, diabetic versus nondiabetic population, treatment duration 1 year or less versus greater than 1 year, RCTs testing different drugs of the same class or different doses of the same drug, addition or not of lifestyle intervention to drug, and for each item of the Cochrane Risk-of-Bias Tool. Publication biases were examined using funnel plots.

Results

The agreement between two reviewers for study selection was 0.88 and for quality assessment of trials was 0.90. The methodological quality of the 49 (30 in NASH) RCTs included was as follows (Fig. 1; Table 1): 19 RCTs had a quality score less than 6, six had a quality score of 6, 24 had a score of 7 or greater (low

Table 1. Characteristics of Randomized Controlled Trials Included in the Analysis

Author	n	Age (yr)	M% BMI	T2DM (%)	Design Quality Score ²	Agent (Daily Dose)	Duration	Liver Enzymes	Liver Fat by Imaging	Histology	Response	
											Comments	Comments
Weight Loss by Dietary Intervention												
Kirk 2009 ¹²	22	44	18	36.5	0 (63% IGT)	RCT 3 (b, c, d, e, h, i)	1000 kcal restriction, as low (10%) CHO or high (65%) CHO	11 wk	Unchanged	Reduced (MRS) by ≈ 40% (NS)	NA	NAFLD 7% weight loss in both arms after 6 weeks; greater improvement in hepatic insulin sensitivity with low CHO
Weight Loss by Lifestyle Intervention (Diet + Exercise)												
Kugelmas 2003 ⁵⁰	16	48	44	33.9	NR	RCT 4 (b, c, d, e, f, i)	AHA step 1 diet+exercise(walking or jogging 30 min/day), with or without vit E 800 IU	12 wk	Improved, together with plasma hyaluronic acid (marker of hepatic fibrosis)	NA	NA	NASH Mean weight loss of 5%(mostly in first 6 weeks); no additional benefit with vitamin E; arms combined for outcome analysis
Promrat 2009 ⁴	41	48	71	34	48%	RCT HQ 7 (b, c)	Intensive lifestyle intervention versus standard counseling Intensive intervention = diet: 1000-1200 kcal/d if baseline weight <91kg, 1200-1500/day if weight >91 kg, fat 25% calories. Physical activity 200 min/week of moderate intensity	48 wk	Improved	-	Improved steatosis and NAS	NASH; lifestyle intervention similar to Look AHEAD) Weight loss: 8.7% vs. 0.5% IBW Dropout rate: 3%
Nobili 2008 ⁵	53	12	70	25.2	2	RCT HQ 9	Lifestyle alone versus lifestyle and antioxidants Diet: 25-30 kcal/kg for overweight subjects; CHO 50-60% fat 23-30%(1/3 SFA) Exercise: 45 min/day moderate aerobic exercise	24 mo	Improved (NS)	NA	Improved steatosis, necroinflammation and NAS(NS)	NAFLD (81% NASH), Significant and similar histological improvement in both arms. Weight loss: 10.7% versus 6.6% IBW
Lazo 2008 ⁷	103	61	49	35	100	RCT 6(b, c, d)	Intensive lifestyle intervention	12 mo	Unchanged	Improved (MRS)	NA	NAFLD; Ancillary study of the Look AHEAD Weight loss: 8.2% versus 0.1% IBW
Weight Loss by Lifestyle + Pharmacological Intervention												
Harrison 2009 ⁶	50	47	32	36.4	10	RCT HQ 4(b, c, d, e, i)	Orlistat 120 mg TID+1400 kcal/d diet versus diet alone (140 kcal/d)	36 wk	Improved (NS)	NA	Improved steatosis, necroinflammation and NAS (NS)	NASH; Vitamin E 800 IU/d added to both arms; weight loss: 8.3% (orlistat) versus 6% (diet); similar improvements in both arms; dropout rate: 18%
Zelber-Sagi 2006 ⁸	52	48	43	33	21	RCT 8 (i)	Orlistat 120 mg TID+weight loss program versus weight loss alone	6 mo	Improved	Improved (US)	Nonsignificantly improved in the 22 follow-up biopsies.	NAFLD, Diet: 104.5 kj/d for IBW, fat<=30% calories Physical activity: 40 min walking at 5-6 km/h 3-4 times/week Dropout rate: 15% Mean weight loss: 8% IBW (orlistat) versus 6% (placebo)

Table 1. Continued

Author	n	Age (yr)	M% BMI	T2DM (%)	Design Quality Score ²	Agent (Daily Dose)	Duration	Response			Comments
								Liver Enzymes	Liver Fat by Imaging	Histology	
Physical Exercise											
Bonekamp 2008 ⁹	45	58	64	31.4	100	RCT 6 (c, d, i)	6 mo	Improved	Improved (MRS)	NA	NAFLD; No significant change in BMI, total or visceral abdominal fat (MRS) Exercise training program: 45' of moderate-intensity aerobic exercise + weight lifting 3 times per week
St George 2009 ¹¹	141	48	62	31.7	NR	RCT 3 (b, c, d, e, h, i)	3 mo	Improved	NA	NA	NAFLD Patients increasing physical activity to ≥150 min/wk (>60% of total patients) improved liver and metabolic outcomes, independently of weight changes. Dietary habits were not reported
Johnson 2009 ¹⁰	23	48	65	31.7	NR	RCT 5 (c, d, h, i)	4 wk	Unchanged	Improved(MRS)	NA	NAFLD Compliance 87% Body weight unchanged; visceral adipose tissue significantly reduced by exercise.
Insulin Sensitizers: Thiazolidinediones											
Sanyal 2004 ¹³	20	46	50	31.6	0	RCT HQ 4 (b, c, d, e, i)	6 mo	Improved (normalized in 95% patients) (NS)	NA	Improved steatosis and inflammation; fibrosis NS	NASH; Vit E 400 IU/d added in both arms; weight change: +0 versus +0.5 kg Dropout rate: 10%(5% for possible drug side effects)
Belfort 2006 ¹⁴	55	51	45	33.2	48%	RCT HQ 7 (h, i)	6 mo	improved	Improved (mean LF reduction of 54% by MRS)	Steatosis and inflammation significantly improved; fibrosis improved (NS)	NASH; Calorie restriction of 500 kcal in both arms Weight change: +2.7% (PIO) versus -1% (placebo) Dropout rate: 13% (7 pts withdrawn 1 for fatigue + low extremity edema)

Table 1. Continued

Author	n	Age (yr)	M%	BMI	Diabetes (%)	Design Quality Score	Agent (Daily Dose)	Duration	Response			Comments
									Liver Enzymes	Liver Fat by Imaging	Histology	
Ratziu 2008 ¹⁶	63	54	59	31	31	RCT HQ 8 (h)	Rosiglitazone 8 mg	12 mo	Improved (normalized in 38%)	NA	Steatosis significantly improved; improve necroinflammation, NAS and fibrosis(NS)	NASH; Weight change +1.5 (rosi) versus -1 (placebo) kg; No lifestyle change was implemented in either arm—absence of diabetes and higher steatosis and serum adiponectin predicted response to treatment (50% patients); dropout rate in ROSI arm: 3%
Aithal 2008 ¹⁵	74	54	61	30.5	0	RCT HQ 8 (h)	Pioglitazone 30 mg	12 mo	Improved	NA	Steatosis improved (NS), hepatocellular injury and fibrosis significantly improved	NASH; Lifestyle intervention (500 kcal restriction + modest exercise 30-40 min 5 days/week) in both arms; mean weight change +3% in PIO versus -4% in placebo arm); Dropout rate in PIO arm: 17.5%
Sanyal 2009 ²³	247	NR	NR	NR	0	RCT HQ 9 3-arm: pioglitazone, vitamin E, placebo	Pioglitazone 30 mg	24 mo	Improved	NA	Improved steatosis and inflammation (NS vs. vitamin E)	NASH; Lifestyle advice in all arms; improved insulin resistance; weigh change: +4.7 kg; primary endpoint met in 34% (P = 0.04 versus placebo)
Torres 2009 ²⁹	49	49	56	31	41	Open-label 3-arm RCT 5(b, c, d, i)	Rosiglitazone 8 mg/d +Metformin 1000 mg	12 mo	Improved	NA	Improved steatosis and inflammation	NASH; weight change: -1.3%
							Rosiglitazone 8 mg +Metformin 1000 mg	12 mo	Improved (NS vs ROSI alone)	NA	Improved steatosis and inflammation (NS versus ROSI alone)	Weight change: -3.3%

Table 1. Continued

Author	n	Age (yr)	M%	BMI	Diabetes (%)	Design Quality Score	Agent (Daily Dose)	Duration	Response			Comments
									Liver Enzymes	Liver Fat by Imaging	Histology	
Omer 2009 ³⁰	64	49	55	30.6	70%	RCT HQ 4 (b, c, d, e, i)	Metformin 1700 mg	12 mo	Improved (NS versus ROSI alone)	NA	Improved steatosis and inflammation (NS versus ROSI alone)	Weight change: -0.5%
							Rosiglitazone 8 mg +losartan 50 mg					
									Unchanged	NA	NAS unchanged	NASH; weight change: -10%
							Rosiglitazone 4 mg	12 mo	Improved	NA	NAS improved	Nonsignificant weight change
							Combination of both	12 mo	Improved	NA	NAS improved (NS versus rosiglitazone)	Weight change: -4%
Tiikkainen 2004 ³¹	20	48	35	30.6	100	RCT 7 (h, i)	Rosiglitazone 8 mg	16 wk	improved	Improved (mean LF reduction of 51% by MRS)	NA	NAFLD; Metformin 2 g in controls ineffective on liver fat; rosiglitazone increased serum adiponectin by 123% and body weight by 0.6 kg
Insulin Sensitizers: Metformin												
Uygun 2004 ²⁴	36	41	62	29.2	0	RCT HQ 4 (b, c, d, e, i)	Metformin 1.5 g	6 mo	Improved	Improved (US)	Improved (NS)	NASH; Diet in both arms; mean weight loss: 6.6% versus 7.9% (NS)
Idilman 2008 ¹⁷	74	47	59	31.5	NR	RCT HQ 3 (b, c, d, e, h, i)	Rosi 8 mg Metformin 1.7 g	12 mo	Improved (NS)	NA	Steatosis and NAS improved	NASH; 20% hyperlipidemic
Haukeland 2009 ²⁵	48	47	73	30.8	27 (48% IGT)	RCT HQ 8 (h)	Diet+exercise Metformin 2.5-3 g	6 mo	Improved (NS)	Unchanged	Unchanged	NASH; lifestyle intervention in both arms; mean weight change: -4.4%(metform) vs. +0.3%
Shields 2009 ²⁶	19	47	68	32.6	0	RCT HQ 8 (l)	Metformin long-acting 0.5-1 g	12 mo	Improved (NS)	NA	Improved (NS)	NASH diet + aerobic exercise in both arms; weight loss: 5% (placebo) versus 3% (metformin)

Table 1. Continued

Author	n	Age (yr)	M% ^a	BMI	Diabetes (%)	Design Quality Score	Agent (Daily Dose)	Duration	Response			Comments
									Liver Enzymes	Liver Fat by Imaging	Histology	
Bugianesi 2005 ²⁷	110	43	85	28.8	7	RCT 6 (b, c, d)	Metformin 2 g versus diet versus vit E 800 IU	12 mo	Improved	NA	Improved steatosis, necroinflammation and fibrosis ¹	NAFLD 6.9% weight loss with both metformin and diet; HOMA improved more consistently with metformin
Nar 2008 ²⁸	34	47	26	32.3	100	RCT 4 (b, c, d, e, i)	Metformin 1.7 g	6 mo	Improved (NS)	Improved (US) (NS)	NA	NAFLD; lifestyle intervention in both arms; mean weight loss: 7% in both arms
Lipid-Lowering Drugs: PUFA												
Spadaro 2008 ³³	40	51	50	30.6	NR	RCT 4 (b, c, d, e, h)	PUFA 2 g	6 mo	Improved	Improved in 50%, reversed in 33% (US)	NA	NAFLD: AHA diet with 25-30 kcal/kg/day in both arms; Dropout rate 10% Mean weight loss: -5.9(PUFA) versus -3.2% (contr.)
Zhu 2008 ³⁴	144	45	72	26.2	NR	RCT 4 (a, b, e, h, i)	PUFA 6 g	6 mo	Improved	Improved in 53%, reversed in 20% (US)	NA	NAFLD with mixed dyslipidemia; AHA diet with 25-30 kcal/kg/day in both arms; dropout rate 7%; weight change not reported
Cussons 2009 ³⁵	25	33	0	34.8	4	RCT 6 (a, e, h)	ω 3-PUFA 4 g	2 mo	Unchanged	Improved (MRS)	NA	NAFLD with PCOS, mostly hyperlipidemic
Lipid-Lowering Drugs: Fibrates												
Basarano-glu 1999 ³⁶	46	43	80	28.2	NR	RCT 5 (b, c, d, h)	Gemfibrozil 600 mg	4 wk	Improved	NA	NA	NASH; no weight changes in either arms; response independent of baseline serum triglycerides
Lipid-Lowering Drugs: Statins												
Nelson 2009 ⁴⁰	16	53	69	36	44	RCT HQ 5 (a, b, h, i)	Simvastatin 40 mg	12 mo	Improved (NS)	NA	Unchanged	Hyperlipidemic NASH dropout rate 13% (no adverse events); no lifestyle intervention

Table 1. Continued

Author	n	Age (yr)	M%	BMI	Diabetes (%)	Design Quality Score	Agent (Daily Dose)	Duration	Response			Comments
									Liver Enzymes	Liver Fat by Imaging	Histology	
Athyros 2006 ⁴¹	63	60	65	32	47 (IFG)	RCT 8 (i)	Atorvastatin 20 mg+ lifestyle advice	12 mo	Improved (normalized in 78%)	Improved (normalized in 67%) (US)	NA	NAFLD and hyperlipidemia in all arms; lifestyle Advice: walking for 30 min, 5 days a week) + NCEP ATP III 500-calorie reduction diet mean weight change: -13%; dropout rate 2%
	62	61	63	32	46(IFG)		Micronized fenofibrate 200 mg+ lifestyle advice		Improved (normalized in 56%)	Improved (normalized in 42%)		Mean weight change: -13% Dropout rate 0%
	61	59	66	33	48(IFG)		Both drugs+ lifestyle advice		Improved (normalized in 78%)	Improved (normalized in 70%)		Mean weight change: -11% dropout rate 3%
Lipid-Lowering Drugs: Probuco												
Merat 2003 ⁴³	30	36	78	28.8	0	RCT 8 (i)	Probuco 500 mg	12 mo	Improved (normalized in 50%)	NA	NA	Normolipidemic NASH; body weight unchanged and HDL-C reduced by 35%; dropout rate 10%
Ursodeoxycholic Acid (UDCA)												
Santos 2003 ⁴⁷	30	37	93	30	NR	RCT 6 (e, h, i)	UDCA 10 mg/kg	3 mo	Improved	Unchanged (CT)	NA	NAFLD Dropout rate: 6%
Mendez-Sanchez 2004 ⁴⁸	27	39	0	33.8	NR	RCT 7 (e, i)	UDCA 1.2 g+1200 kcal diet versus diet alone	6 wk	Improved (NS)	Improved (US)(NS)	NA	NAFLD Weight loss: 7% (UDCA) versus 8% (diet) Dropout rate: 11%
Ersöz 2005 ⁴⁹	57	47	59	28.6	25	RCT 3 (b, c, d, e, h, i)	UDCA 10 mg/kg versus vit E 600 IU + vitamin C 500 mg	6 mo	Improved (NS)	Unchanged (US)	NA	NAFLD Similar between-arm changes in liver enzymes

Table 1. Continued

Author	n	Age (yr)	M%	BMI	Diabetes (%)	Design Quality Score	Agent (Daily Dose)	Duration	Response			Comments
									Liver Enzymes	Liver Fat by Imaging	Histology	
Lindor 2004 ⁴⁴	166	47	44	32	NR	RCT HQ 8 (h)	UDCA 13-15 mg/kg	2 yr	Improved (NS)	NA	Improved (NS)	NASH; Similar between-arm changes in liver enzymes and histology dropout rate: 24%
Dufour 2006 ⁴⁵	48	46	65	30	21	RCT HQ 8 (i)	UDCA 12-15 mg/kg+ vitamin E 800 IU	2 yr	Improved (normalized in 53% patients)	NA	Steatosis improved vs. placebo + placebo	NASH; weight unchanged in all arms; dropout rate: 16%
Ratziu 2009 ⁴⁶	126	50	75	30.9	35	RCT 8(i)	UDCA + placebo Placebo + placebo UDCA 28-35 mg/kg	1 yr	Improved Unchanged Improved	NA NA NA	Unchanged Unchanged NA	NASH; adverse effects of UDCA: diarrhea (45%), abdominal pain (31%)
Antioxidants												
Rui 2001 ⁵¹	47	53	41	29	NR	RCT 3 (a, b, c, d, e, i)	Reduced glutathione (1200 mg) IV	2-3 wk	Improved (NS)	NA	NA	NASH
Pamuk 2003 ⁵⁴	35	49	53	28.1		RCT 4 (b, c, d, e, i)	N-acetylcysteine 600 mg	4 wk	Improved (NS)	NA	NA	NASH
Kugelmas 2003 ⁵⁰	16	48	44	33.9	NR	RCT 5 (c, d, f, i)	Vit E 800 IU	12 wk	Improved, together with plasma hyaluronic acid (NS)	NA	NA	NASH Mean weight loss of 5% (mostly in first 6 weeks); AHA step 1 diet + exercise (walking or jogging 30 min/day) in both arms
Harrison 2003 ⁵²	49	51	56	32.7	42	RCT HQ 9	Vit E 1000 IU+vit C 1000 mg plus weight-loss counseling	6 mo	Unchanged	NA	Improved fibrosis (NS)	NASH; Dropout rate: 8% Nonsignificant improvement in fibrosis compared with placebo
Abdelmalek 2005 ⁵⁵	55	44	40	33.6	10	RCT HQ 8 (i)	Betaine anhydrous 20 g	12 mo	Unchanged	NA	Unchanged	NASH; dropout rate: 64% for GI intolerance or allergy
Dufour 2006 ⁴⁵	48	46	65	30	21	RCT HQ 8 (i)	UDCA 12-15 mg/kg+ vitamin E 800 IU	2 yr	Improved (normalized in 53% patients)	NA	Steatosis improved	NASH
Nobili 2008 ⁵	53	12	70	25.2	2	RCT HQ 9	Vitamin E 600 IU + vitamin C 500 mg	24 mo	Improved (NS)	NA	Improved steatosis, necroinflammation and NAS (NS)	NAFLD (81% NASH), Significant and similar histological improvement in both arms. Weight loss: 10.7% versus 6.6% IBW

Table 1. Continued

Author	n	Age (yr)	M%	BMI	Diabetes (%)	Design Quality Score	Agent (Daily Dose)	Duration	Response			Comments
									Liver Enzymes	Liver Fat by Imaging	Histology	
Sanyal 2009 ²³	247	NR	NR	NR	0	RCT HQ 9 3-arm: vitamin E, pioglitazone, placebo	Vitamin E 800 IU	24 mo	Improved	NA	Improved steatosis and inflammation (NS vs. pioglitazone)	NASH; Lifestyle advice in all arms; weight change: +0.4 kg primary endpoint met in 43% (P = 0.001 versus placebo)
Miglio 2000 ⁵⁵	191	57	70	29	14	RCT 8(i)	Betaine glucuronate 300 mg	8 wk	improved	Improved (US)	NA	NAFLD; dropout rate 0%
Ersoz 2005 ⁴⁹	57	47	59	28.6	25	RCT 3 (b, c, d, e, h, i)	UDCA 10 mg/kg versus vit E 600 IU + vitamin C 500 mg	6 mo	Improved (NS)	Unchanged (US)	NA	NAFLD Similar between-arm changes in liver enzymes
Bugianesi 2005 ²⁷	110	43	85	28.8	7	RCT 6 (b, c, d)	Vit E 800 IU	12 mo	Improved (NS)	NA	NA	NAFLD
Vilar Gomez 2009 ⁵⁶	60	47	57	30	0	RCT HQ 6 (b, c, d)	Viusid 50 g	6 mo	Improved (NS)	NA	Steatosis and necroinflammation improved in both arms	NAFLD; both arms had diet + exercise and lost 11% body weight; dropout rate 18%
Anti TNF-α Agents (Pentoxifylline)												
Buranawui 2007 ⁵⁸	32	49	59	26.5	15	RCT 5 (b, c, d, i)	Pentoxifylline 1200 mg versus placebo	6 mo	Improved	NA	NA	NASH Dropout rate 3% (for nausea) diet in both arms
Lee YM 2008 ⁵⁹	20	47	65	28	10	RCT 7 (h)	Pentoxifylline 1200 mg versus placebo	3 mo	Improved	NA	NA	NASH; AHA Step I diet in both arms
Rinella 2009 ⁶⁰	25	NR	NR	NR	NR	RCT HQ 8(i)	Pentoxifylline 1200 mg	12 mo	Improved	NA	Steatosis improved (NS), ballooning and NAS improved	NASH Dropout rate: 16% (0% for adverse effects)
Antihypertensive Drugs												
Georgescu 2009 ⁶¹	54	49	52	27.4	FPG <130 mg/dL without any treatment	RCT HQ 7 (h, i)	Telmisartan 20 mg versus valsartan 80 mg	20 mo	Improved	NA	Steatosis, necroinflammation and fibrosis improved with telmisartan	Hypertensive NASH; no weight changes; improved insulin sensitivity in both arms (greater with telmisartan) Dropout rate 0%

Table 1. Continued

Author	n	Age (yr)	M%	BMI	Diabetes (%)	Design Quality Score	Agent (Daily Dose)	Duration	Response			Comments
									Liver Enzymes	Liver Fat by Imaging	Histology	
Endocannabinoid Receptor Antagonists												
Despres 2008 ⁶²	231	49	46	36	NR	RCT 7 (e, i)	Rimonabant 20 mg	1 yr	Improved	Improved (CT)	NA	NAFLD 600 kcal/day caloric restriction in both arms. Weight loss higher with drug: 5.8% versus 2% IBW Dropout rate for adverse effects: 14.1% (drug) versus 10.1% (placebo)
L-Carnitine												
Malaguar- nera 2010 ⁶³	74	48	54	26.6	26%	HQ RCT 9	L-carnitine 1 g	6 mo	Improved	NA	Improved steatosis and NAS	Lifestyle intervention in both arms; weight loss: 4% (controls) versus 5% (treatment); drop-out rate 0%

For each treatment, trials on biopsy-proven NASH are grouped together and presented before trials on patients with NAFLD. The trial design is followed in the same box by the Cochrane Risk-of-Bias Tool score for RCTs (score range: 0-9).

Quality items of RCTs according to Cochrane Risk-of-Bias Tool (score range, 0-9):

- A: Adequate method of sequence generation.
- B: Blinding of participants performed.
- C: Blinding of personnel performed.
- D: Blinding of assessors performed.
- E: Allocation concealment adequate.
- F: Adequate assessment of each outcome.
- G: Selective outcome reporting avoided.
- H: Intention-to-treat analysis of results.
- I: Sample size/power calculation reported.

¹Post-treatment biopsy available only for 17 patients in the metformin arm.

²For RCTs comparing two different lifestyle interventions, blinding of participants and personnel was not applicable: therefore, quality score ranges 0-7.

Abbreviations: T2DM, type 2 diabetes mellitus; NS, no significant difference in improvement between treatment and control group at the end of treatment; LF, liver fat; NR, not reported; NAC, N-acetylcysteine; NA, not assessed; BW, body weight; IBW, ideal body weight; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; AHEAD, Action for Health in Diabetes; AHA, American Heart Association; HQ, high-quality RCT; HR, heart rate; HRQL, health-related quality of life; PUFA, polyunsaturated fatty acids; CHO, carbohydrates; HP EPA, highly purified eicosapentaenoic acid; IFG, impaired fasting glycemia (fasting plasma glucose: 100-125 mg/dL); IGT, impaired glucose tolerance; DASH, Dietary Approaches to Stop Hypertension; PCOS, polycystic ovary syndrome.

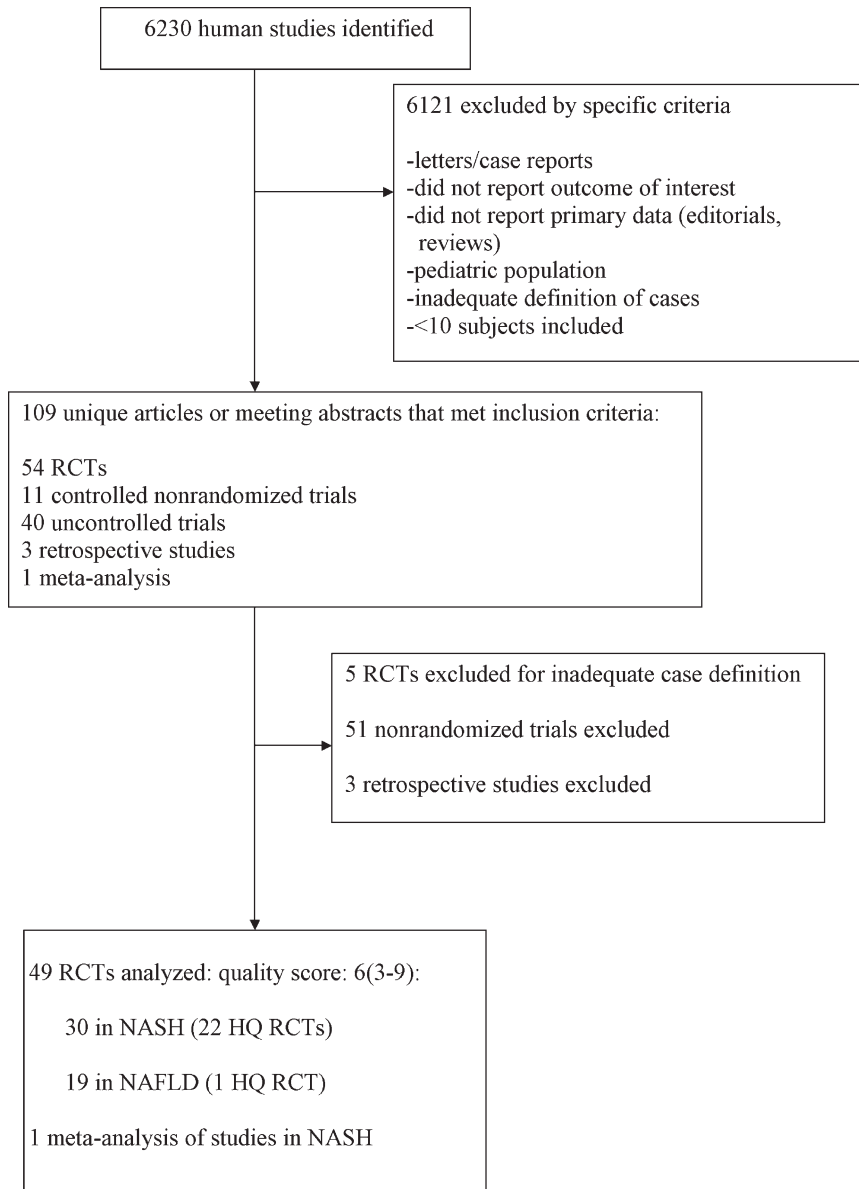


Fig. 1. Evidence acquisition flow diagram. Quality scores of included studies are included as median, range. High quality (HQ) RCTs are RCTs with post-treatment histology.

risk of bias). Allocation concealment and double-blinding were adequate in 45% and in 69% of RCTs; an intention-to-treat analysis was performed in 69% of RCTs. Sample size was calculated *a priori* in 4 of 19 RCTs with negative results.

Twenty-two RCTs in NASH and 1 RCT in NAFLD had post-treatment histology and were defined as high quality (HQ); RCTs in NASH were examined separately from trials in NAFLD, which may include patients with simple steatosis and with NASH.

Weight Reduction Through Lifestyle and Pharmacological Intervention

Trials in NASH. Three HQ RCTs (total 125 participants; two RCTs with quality score ≥ 7) were included.

The RCT by Promrat randomized 41 overweight patients to 1 year of an intensive lifestyle intervention program, similar to that implemented in the Look AHEAD, or standard nutritional counseling.⁴ Compared with standard counseling, the intensive lifestyle intervention arm lost significantly more weight (mean weight loss, 8.7% versus 0.5%), improved steatosis and NAFLD activity score (NAS), a sum of steatosis and necroinflammatory scores indicating overall disease activity, and reversed NASH in 67% of participants (versus 20% of controls, $P = 0.02$). Other histological features did not significantly change. Only those patients (39% of total) losing 7% or more body weight significantly improved histological disease activity score whereas the remaining patients showed nonsignificant histological changes compared with controls.

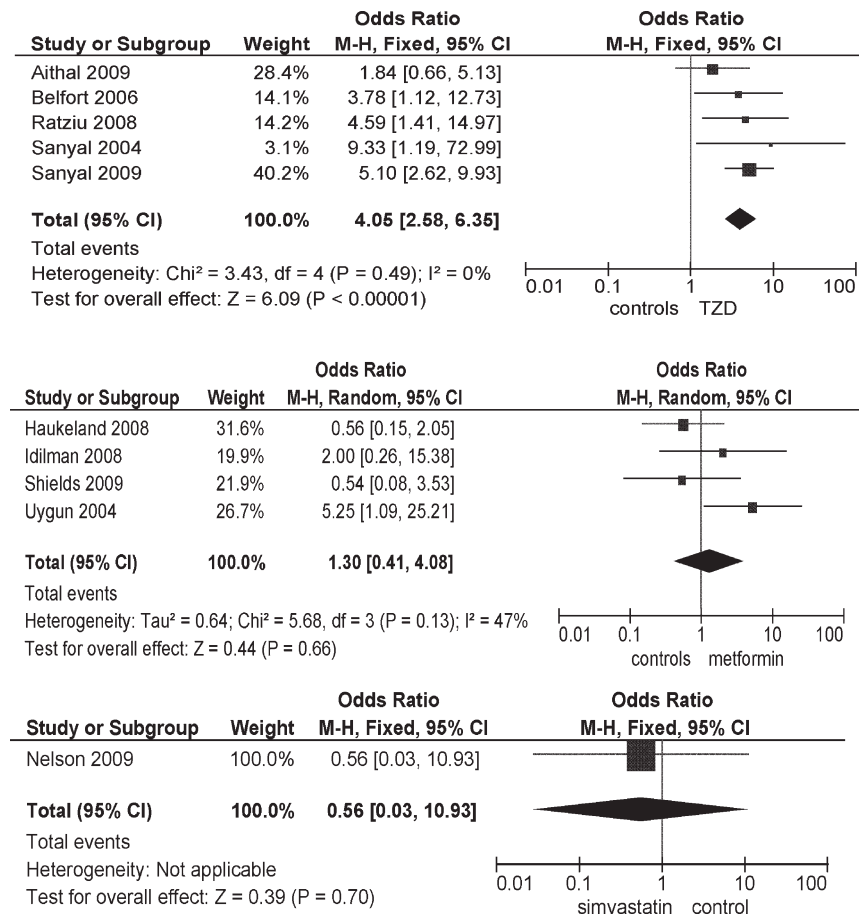


Fig. 2. Forest plot of RCTs comparing the effect of drugs on histological steatosis in NASH.

Another RCT randomized 53 pediatric-adolescent patients to lifestyle intervention alone or with antioxidant vitamins E and C.⁵ After 24 months, weight loss averaged 6.6% and 10.7%, respectively ($P = 0.9$). Steatosis, lobular inflammation, hepatocyte ballooning, and NAS significantly improved in the two arms, and NASH resolved in 71% versus 92% patients ($P = 0.27$), respectively. Homeostasis Model Assessment (HOMA), plasma glucose, and lipids also significantly improved.

The third RCT evaluated lifestyle intervention, alone or with orlistat, in overweight patients.⁶ Compliance to pharmacological treatment exceeded 80%; weight loss averaged 8% with orlistat and 6% with placebo. Both arms significantly and similarly improved steatosis, necroinflammation, and NAS. Patients losing 5% or more weight improved steatosis, insulin resistance, and plasma glucose, but only those subjects (41% of total) losing 9% or more weight also improved necroinflammation and NAS.

These RCTs suggest that weight loss is safe and can improve histological and metabolic parameters in NASH. Whether the required weight loss to improve

histological disease activity is higher than that required to improve steatosis and metabolic parameters needs further confirmation.

Trials in NAFLD

Two RCTs (155 participants, quality score 6, 8) assessed the effect of lifestyle intervention on NAFLD.

In diabetic patients enrolled in the RCT Look AHEAD (abstract), 1 year of intensive lifestyle intervention enhanced weight loss (−8.2% versus −0.1%, $P = 0.009$), slightly improving magnetic resonance spectroscopy (MRS)-detected hepatic steatosis (−3.02% versus −1.45%, $P = 0.003$) and hemoglobin A1c levels compared with standard counseling.⁷

In a placebo-controlled RCTs evaluating 6 months of orlistat plus lifestyle intervention,⁸ weight loss approached that observed by Harrison et al.⁶; however, ALT and ultrasonographic steatosis improved more consistently with orlistat. Orlistat was safe and well tolerated, with minor adverse gastrointestinal complaints not requiring discontinuation of therapy.

Three RCTs (209 participants, quality score ranging 3–6) assessed the effect of *physical activity alone* in

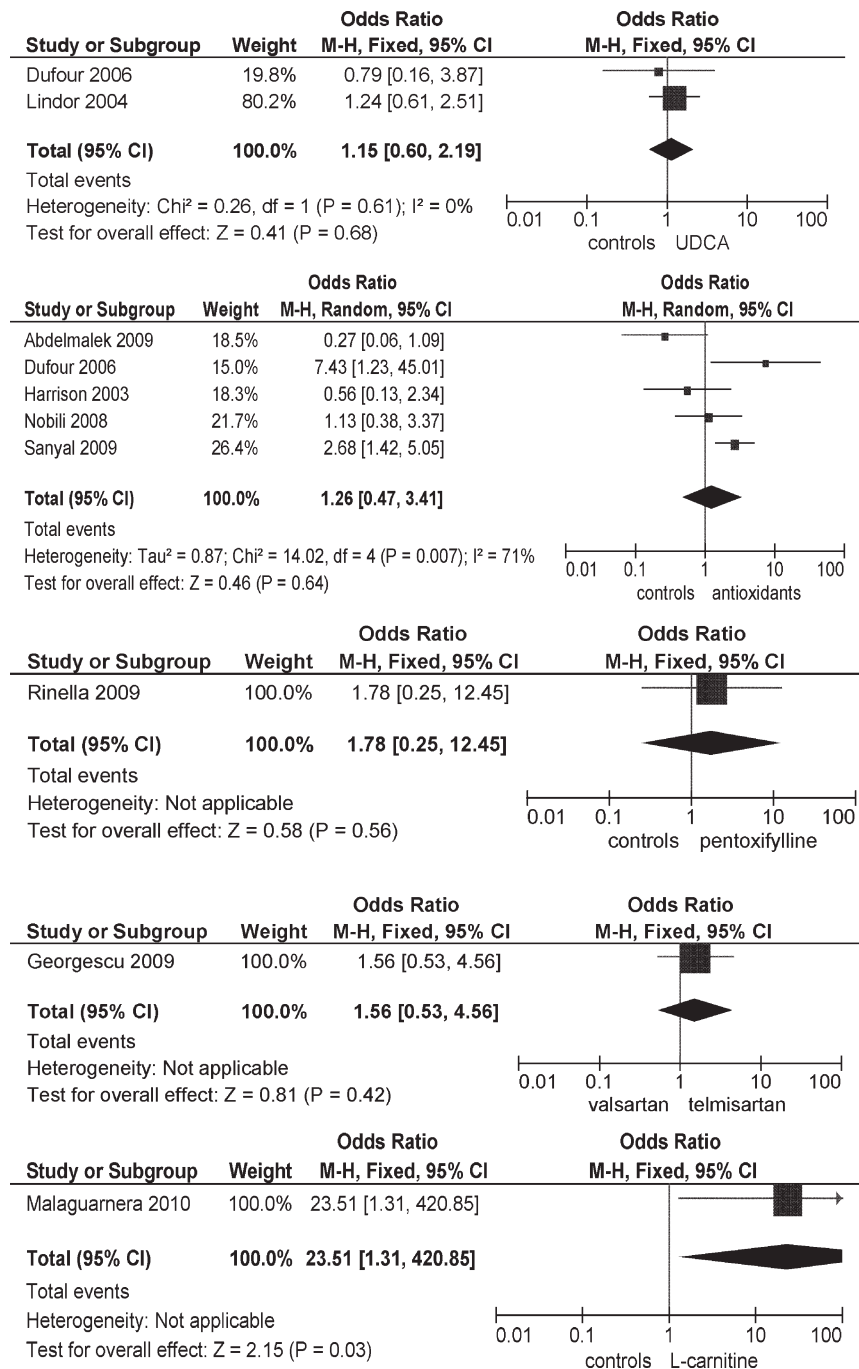


Fig. 2. (Continued)

NAFLD. In the first (abstract), 6 months of moderate-intensity aerobic exercise reduced MRS-detected hepatic (−2.5%, *P* = 0.003 versus controls) and subcutaneous abdominal fat and hemoglobin A1c in obese diabetic subjects, despite comparable changes in body mass index, lean body mass, and total or visceral fat.⁹

In another RCT, 4 weeks of moderate-intensity aerobic exercise reduced MRS-detected hepatic fat by 21% (*P* = 0.04 versus controls) and visceral adipose tissue compared

with controls. Body weight, HOMA, and plasma lipids did not significantly change.¹⁰ Importantly, dietary habits did not change throughout the study.

In the last, 3 months of increased physical activity dose-dependently improved liver enzymes, abdominal obesity, and metabolic abnormalities in 141 overweight subjects, independent of weight changes.¹¹

These 3 RCTs suggest that exercise *per se* improves indices of hepatic steatosis independently of weight

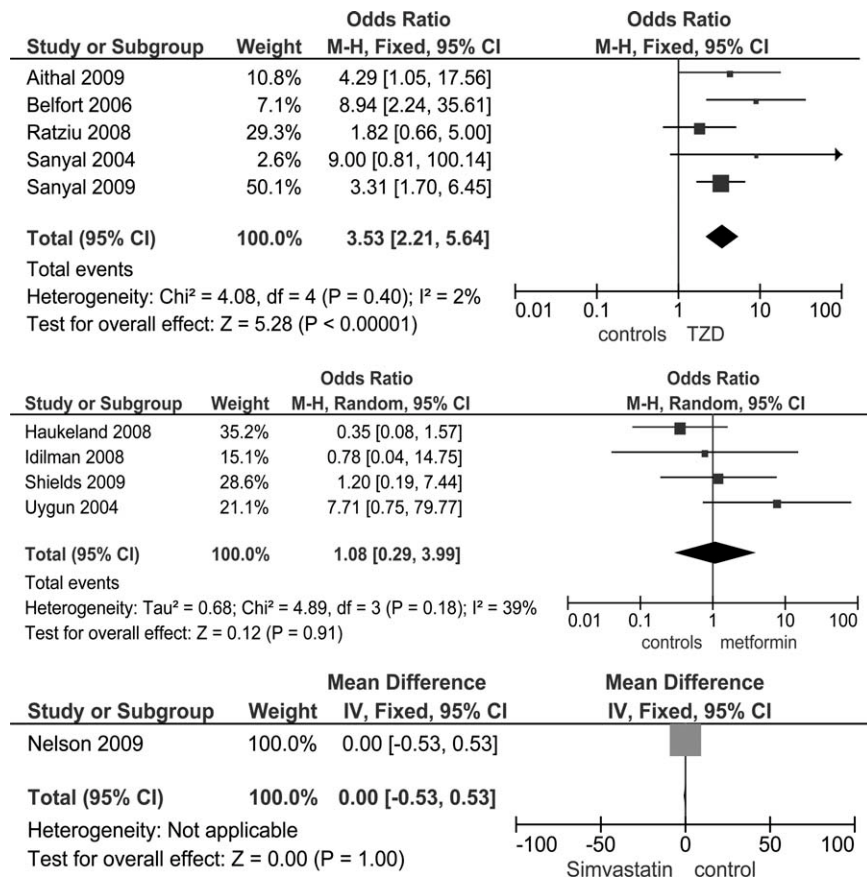


Fig. 3. Forest plot of RCTs comparing the effect of drugs on histological inflammation in NASH.

loss. The optimal type and dose of exercise in NAFLD are currently being investigated in one RCT (clinicaltrials.gov identifier NCT00771108).

Indications on the optimal *nutrient dietary composition* for NAFLD are sparse, deriving mostly from comparative analysis of observational studies. In one RCT enrolling 22 obese glucose-intolerant NAFLD subjects, 11 weeks of a low-carbohydrate caloric restriction induced comparable weight loss (−7%), and hepatic (−42%) and intra-abdominal fat reduction, but improved hepatic insulin sensitivity by nearly threefold compared with a high-carbohydrate isocaloric reduction.¹² This RCT suggests that caloric restriction is the most important goal for improving hepatic steatosis in NAFLD, but carbohydrate restriction may further benefit glucose metabolism in glucose-intolerant patients.

Trials in NASH

Five HQ RCTs (354 participants, 4 RCTs with quality score ≥7) assessed the effect of 6 to 24 months of pioglitazone (four trials) or rosiglitazone (one RCT) on liver histology in NASH.¹³⁻¹⁷ Pooled results of RCTs showed that TZDs improved histological steatosis and inflammation but not fibrosis (Figs. 2-4). Heterogeneity

was low for all assessed outcomes, suggesting a consistent drug effect size across studies. Presence of diabetes, the implementation of lifestyle intervention, different drug dose, or trial duration did not affect results. No significant publication bias was detected.

TZDs consistently improved hepatic, muscle, and adipose tissue insulin resistance,^{13,14,18} and reduced plasma glucose and hemoglobin A1c in glucose-intolerant subjects.¹⁴⁻¹⁶ Pioglitazone lowered plasma triglyceride in glucose-intolerant subjects, but rosiglitazone worsened total and low-density lipoprotein cholesterol.¹⁶ Only one RCT reported the impact of TZDs on blood pressure, with nonsignificant effects.¹⁵

Two RCTs evaluated the predictors of histological response to TZDs: in the Fatty Liver Improvement with Rosiglitazone Therapy (FLIRT), absence of diabetes, a higher baseline serum adiponectin, and severe steatosis predicted histological response.¹⁶ In another RCT,¹⁴ histological response was similar between diabetic and nondiabetic subjects, whereas increased adiponectin levels and adipocyte insulin sensitivity predicted histological improvement.¹⁸

The most common side effects were weight gain of 2-5 kg (66%-75% of patients), and lower extremity

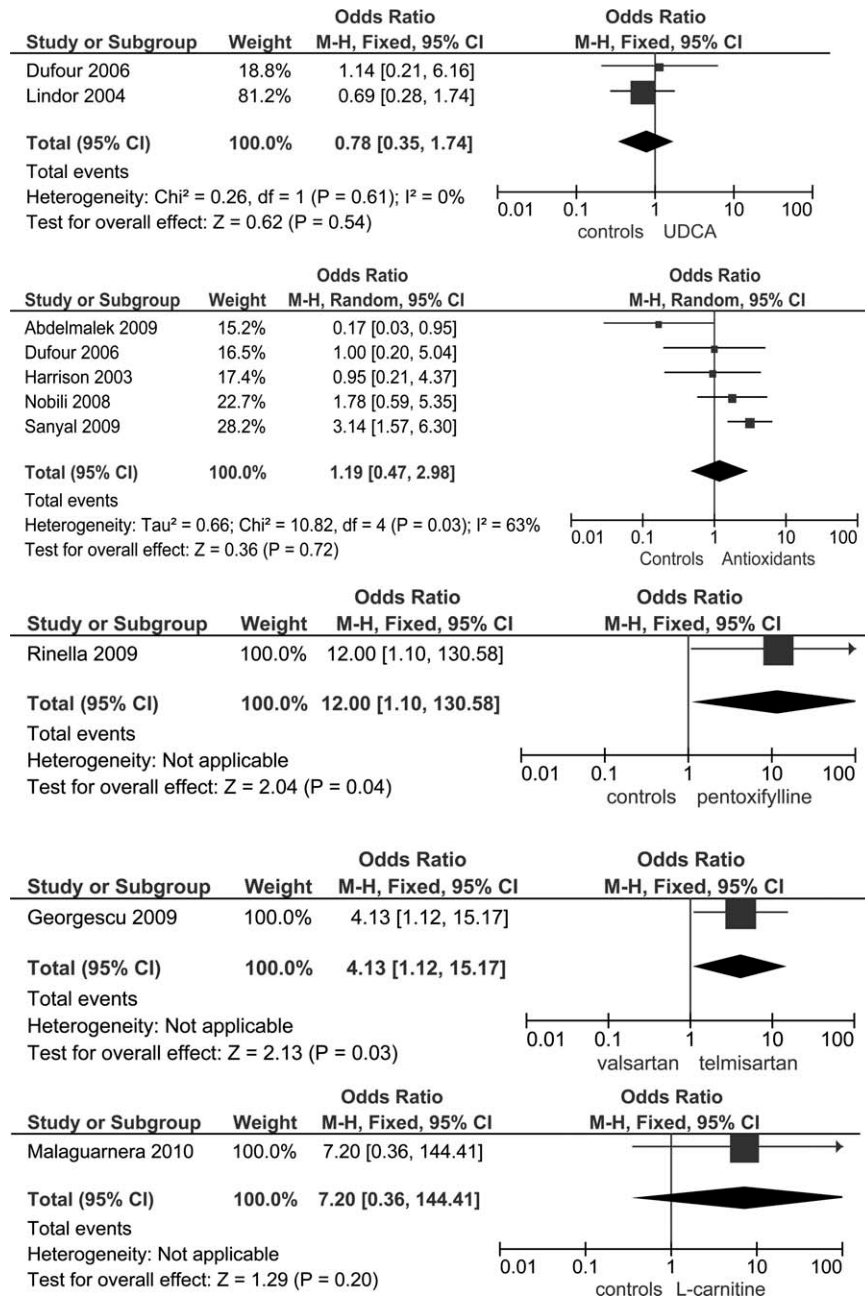


Fig. 3. (continued)

edema (4%-10%), the latter often causing treatment discontinuation. Weight gain occurred despite lifestyle intervention, which was implemented in all but the FLIRT trial. Dropout rate ranged 4%-18% of patients.

Because of the short trial duration, there was no report of congestive heart failure, bone fractures, or increased cardiovascular mortality, but the benefit-safety profile of overall and single TZDs, as well as eventual benefit on hepatic fibrosis, warrant assessment in larger RCTs of longer duration.

The effects of TZD discontinuation were evaluated in an extension of two small uncontrolled trials. In the first, NASH and associated metabolic abnormalities relapsed 1 year after discontinuing pioglitazone.¹⁹ In the second, subjects achieving and maintaining lifestyle changes and weight loss displayed sustained histological and metabolic benefit 37 months after TZD discontinuation.²⁰

The effect of prolonged therapy with TZDs is unknown, because most RCTs lasted 12 or fewer months. Two trials evaluated the effects of TZD

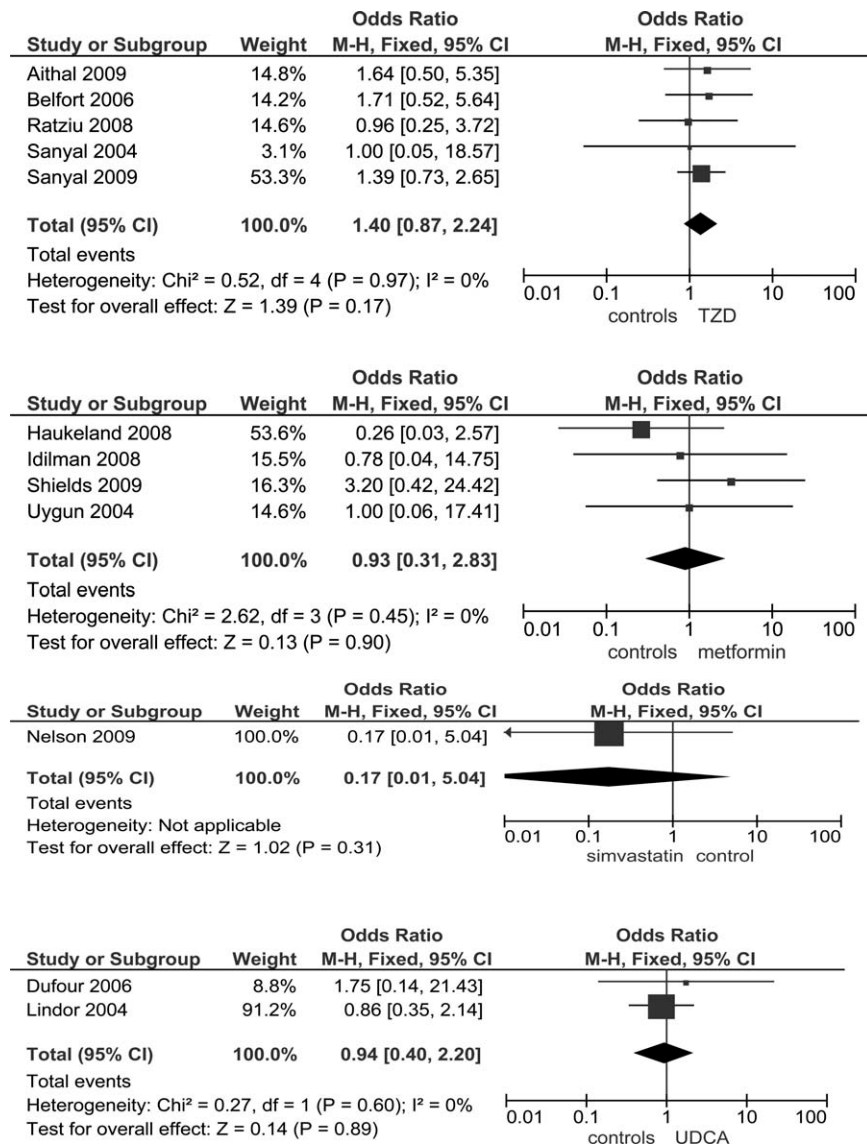


Fig. 4. Forest plot of RCTs comparing the effect of drugs on histological fibrosis in NASH.

treatment for up to 2 and 3 years, respectively. In the open-label FLIRT-2, patients completing the FLIRT trial were placed on rosiglitazone for 2 additional years: despite a continued improvement in insulin sensitivity and aminotransferases, rosiglitazone did not further improve liver histology.²¹ The “Pioglitazone Versus Vitamin E Versus Placebo for the Treatment of Nondiabetic Patients with NASH” trial (abstract at American Association for the Study of Liver Diseases 2009), randomized 247 nondiabetic patients to 2 years of either pioglitazone, vitamin E, or placebo. Lifestyle intervention was implemented in all arms. Compared with placebo, pioglitazone improved insulin sensitivity, steatosis, and inflammation, and reversed NASH in 50% of patients versus 25% of placebo-treated subjects ($P = 0.0008$), but the primary endpoint (NAS improvement by 2 or more points and

no worsening of fibrosis) was achieved by only 34% of patients on pioglitazone versus 19% of patients on placebo ($P = 0.04$).^{22,23}

These trials suggest that long-term therapy with TZDs may be required for sustained histological improvement but offer no additional histological benefit. Furthermore, improving insulin sensitivity may not be sufficient for improving liver injury, and other therapeutic approaches might be warranted for a durable control of disease activity in NASH.

Trials in NASH

In four HQ RCTs (115 participants, two RCTs with quality score >7), 6-12 months of metformin plus lifestyle intervention did not improve liver histology or aminotransferases, compared with lifestyle

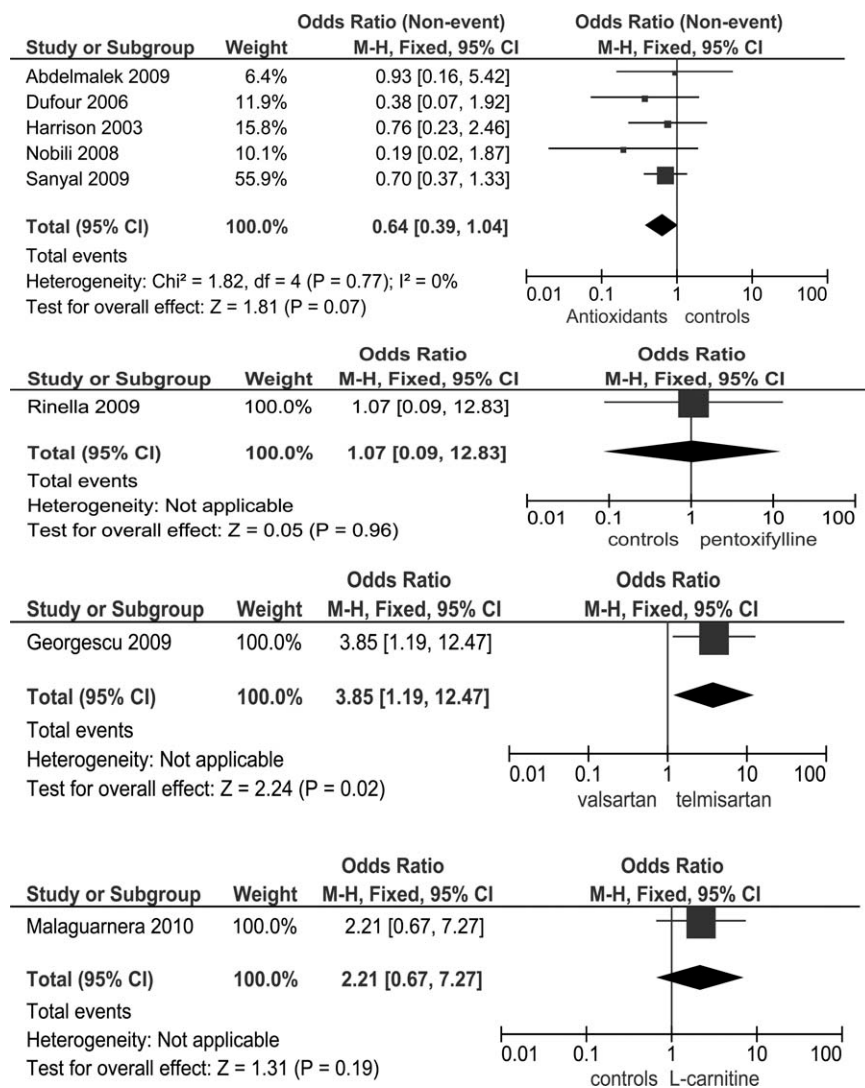


Fig. 4. (continued)

intervention alone, independently of dose, treatment duration, or diabetic state^{17,24-26} (Figs. 2-5). No publication bias was detected.

Trials in NAFLD

Two RCTs (144 participants, quality score <7) evaluated the effect of metformin on radiological and biochemical indices of NAFLD.^{27,28} In one RCT, metformin normalized aminotransferases in 69% versus 31% of the diet group ($P = 0.003$). In the other RCT, biochemical and radiological improvement was nonsignificant compared with diet + exercise.

Overall, when added to lifestyle intervention, metformin enhanced weight loss (mean weight loss, 4.3%-7.9%) and improved insulin sensitivity and plasma glucose levels: the latter effects were most consistent in diabetic patients and outweighed the magnitude of weight loss.^{25,28} The plasma lipid profile did not significantly

change. Metformin was safe and well-tolerated, with gastrointestinal intolerance being the most common adverse effect, not usually requiring discontinuation of therapy. Despite its weight-losing and insulin-sensitizing properties, the effects of metformin on liver histology warrant further evaluation in larger and longer RCTs.

Insulin-Sensitizers: Thiazolidinediones vs. Metformin or vs. a Combination of Both Drugs

Trials in NASH. Two open-label HQ RCT (113 participants, quality score <6, one in abstract form) compared the combination of rosiglitazone plus metformin versus each agent alone in NASH.^{29,30} After 1 year, steatosis and necroinflammation significantly improved with rosiglitazone, but not with metformin; the combination of both enhanced weight loss over rosiglitazone alone but conferred no additional benefit on liver histology and glucose metabolism.

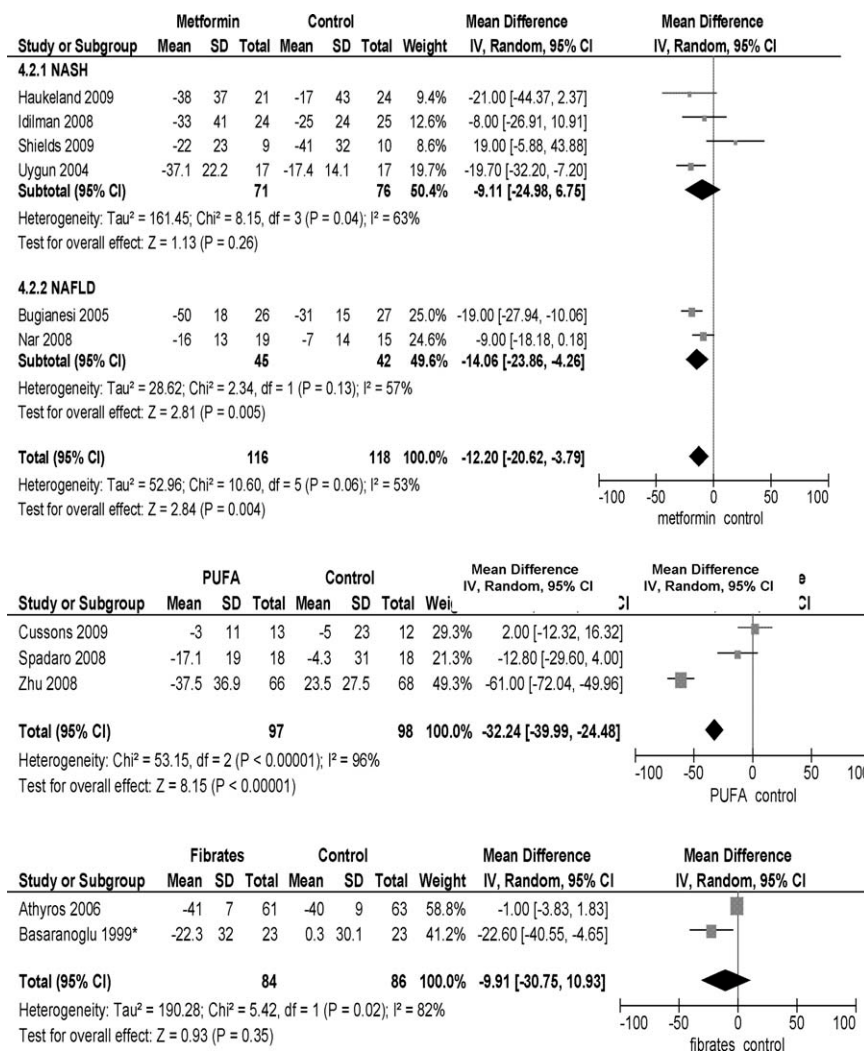


Fig. 5. Forest plot of comparison of RCTs comparing the effects of drugs on ALT levels in NAFLD.

Trials in NAFLD. In a small RCT on diabetic NAFLD patients, rosiglitazone reduced hepatic fat by sevenfold compared with metformin ($P = 0.003$), an effect closely correlating with increased adiponectin levels. Both drugs improved hepatic insulin sensitivity, whereas peripheral insulin sensitivity increased only with rosiglitazone.³¹

Lipid-Lowering Drugs

N-3 Polyunsaturated Fatty Acids (PUFA). In three RCTs (209 participants, quality score < 7), PUFAs ameliorated aminotransferases and radiological steatosis in NAFLD (Fig. 5). Steatosis by MRS³⁵ or ultrasonography^{33,34} resolved in 20%-64% of cases. However, ultrasonography is a qualitative, operator-dependent technique and misses steatosis of lower grade (involving < 33 % hepatocytes), making it hard to compare ultrasonographic with MRS trails.³²⁻³⁵ An improvement in

hypertriglyceridemia and insulin resistance was also observed. Overall, PUFAs were well tolerated, with minor gastrointestinal symptoms. The heterogeneity in population, treatment duration, doses, methods to assess radiological outcomes, and the lack of post-treatment histology prevent any definitive conclusion

Three RCTs (clinicaltrials.gov identifier: NCT-00323414, NCT00681408, NCT00760513) are evaluating PUFA in NASH.

Fibrates

Trials in NASH. In one RCT (quality score 8), fibrates had no significant benefit on histological, biochemical, or radiological outcome.³⁶

Statins

The antioxidant and anti-inflammatory properties, the frequent coexistence of NAFLD and dyslipidemia,

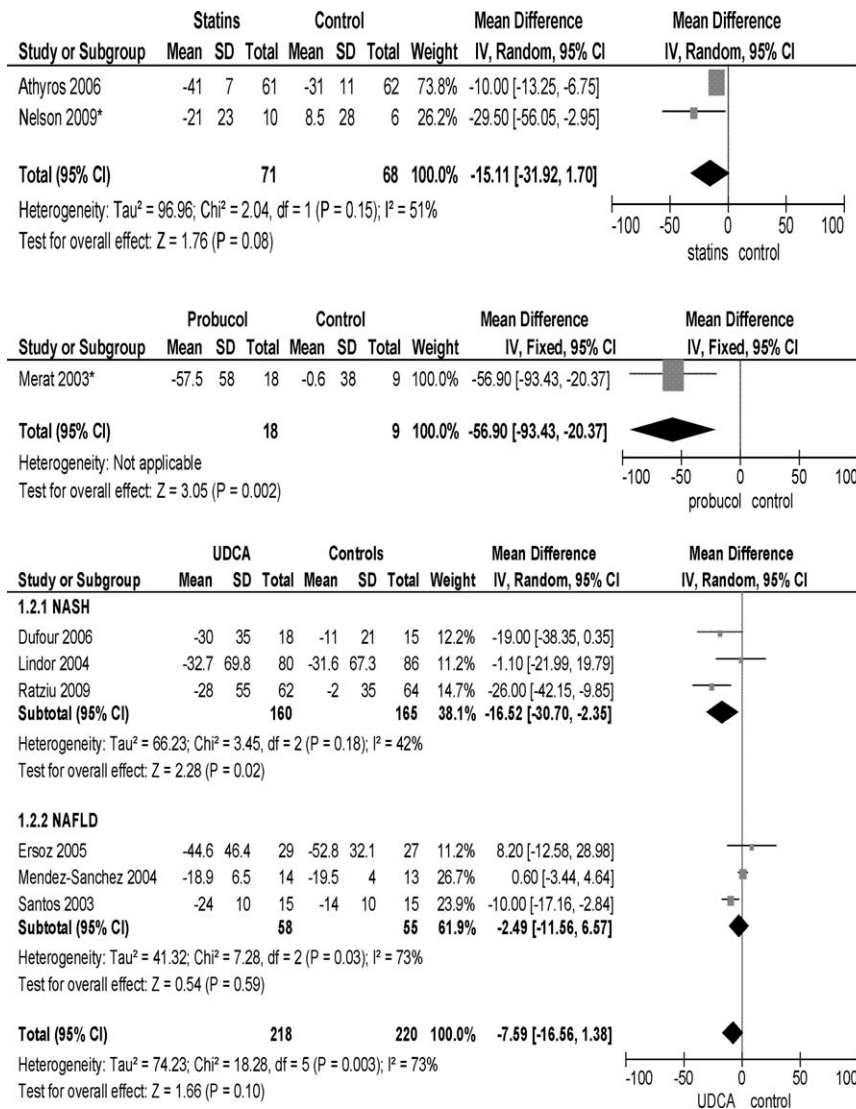


Fig. 5. (continued)

and the increased cardiovascular risk of these patients make statins an attractive therapeutic tool in NAFLD. Despite these premises, data on statin efficacy in NAFLD are sparse because of the feared hepatotoxicity of these drugs. A meta-analysis of 13 large trials showed no increase in liver enzymes with statins.³⁷ Furthermore, patients with hepatic steatosis do not seem at increased risk for statin hepatotoxicity.³⁸ On this basis, the Liver Expert Panel stated that statins can be safely used in patients with NAFLD, and routine liver enzyme monitoring is not warranted in this population.³⁹

Trials in NASH. In a small HQ RCT, 1 year of simvastatin was safe but did not improve liver histology (Table 1; Figs. 2-4).⁴⁰

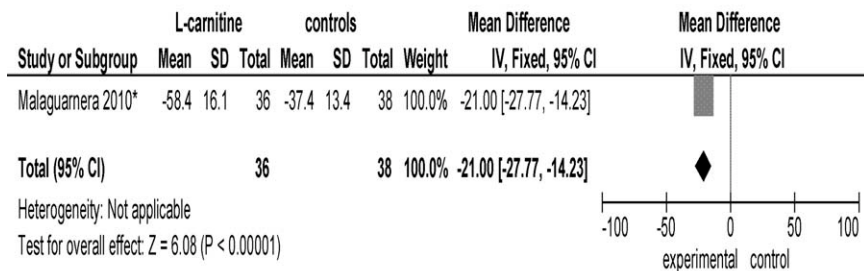
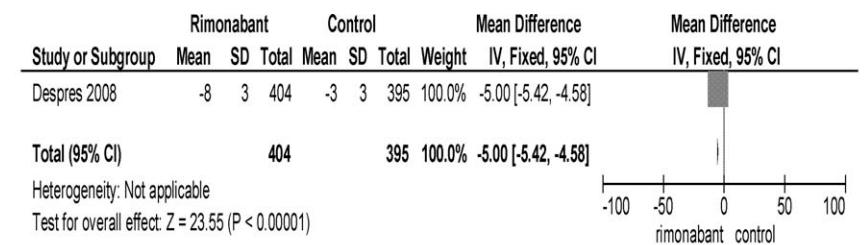
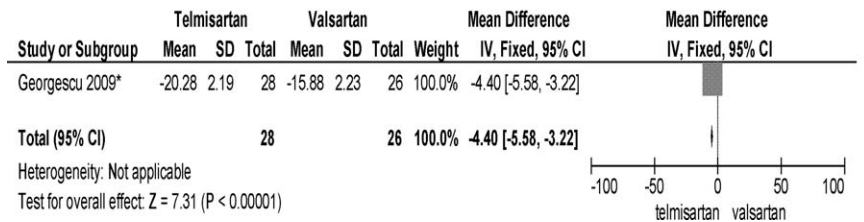
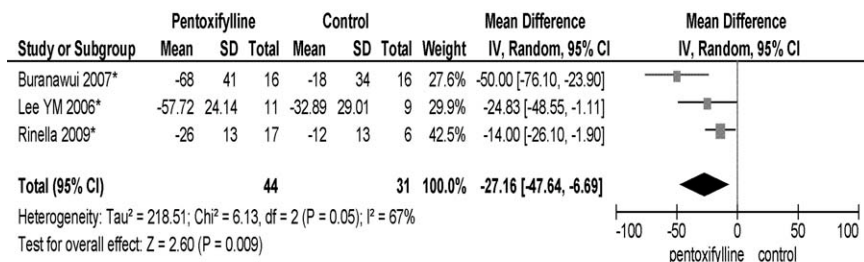
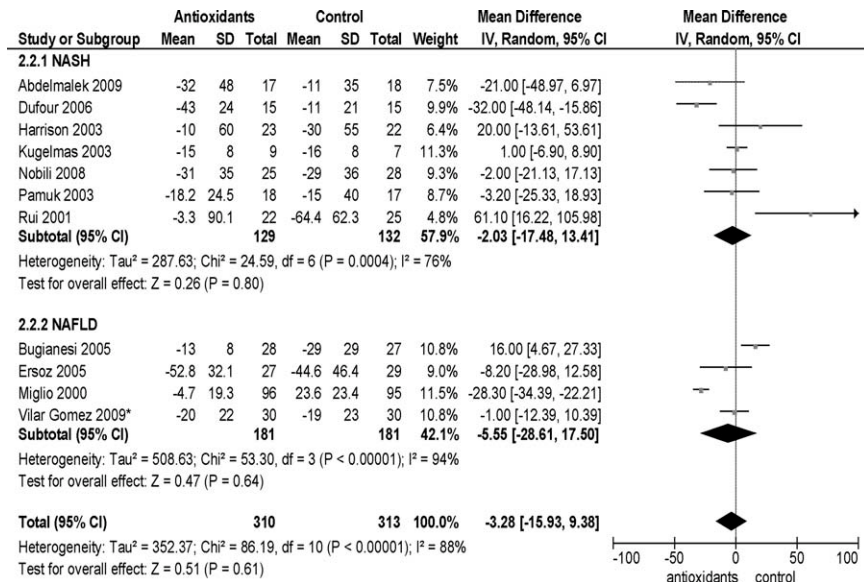
Trials in NAFLD. An RCT (quality score 8) randomized 186 hyperlipidemic NAFLD patients to 12 months of lifestyle advice plus atorvastatin, fenofibrate,

or a combination of both (Table 1; Fig. 5).⁴¹ Despite a consistent weight loss (11-13%) in all arms, biochemical plus ultrasonographic regression of NAFLD was significantly higher with atorvastatin, alone or in combination, than with fenofibrate. Weight loss greater than 4% and concomitant use of orlistat or metformin independently predicted treatment response.

The effects of statin exposure on liver histology over 10-16 years in 68 patients with NAFLD were retrospectively reviewed.⁴² Despite a higher baseline risk profile for liver disease progression, patients on statin improved liver steatosis and slowed fibrosis progression compared with controls.

Probuco

Trials in NAFLD. Probuco, a lipophilic lipid-lowering drug with strong antioxidant activity,



For RCTs with hypolipemizing drugs, pentoxifylline telmisartan/valsartan, L-carnitine

the asterisk* marks RCTs in histologically-proven NASH

Fig. 5. (continued)

significantly improved aminotransferases (with normalization in 50% of patients) in a small RCT (quality score 8).⁴³ Although generally well tolerated, probucol significantly reduced high-density lipoprotein cholesterol levels, raising safety concern in patients at high cardiovascular risk.

In summary, further large, prospective, placebo-controlled trials with histological endpoints are warranted before any firm conclusion can be drawn on the effectiveness of lipid-lowering drugs in NAFLD/NASH.

Ursodeoxycholic Acid

Trials in NASH. Pooled results of three RCTs (340 participants, quality score 8) revealed marginally significant benefit of ursodeoxycholic acid (UDCA) on liver enzymes, the effect being entirely explained by the high-dose UDCA RCT (Fig. 5).⁴⁵⁻⁴⁷ Two of these RCTs assessed post-treatment-histology, finding no benefit over placebo, but the effect of high-dose UDCA on liver histology is unknown (Figs. 2-4; Table 1).

Trials in NAFLD. In three RCTs (113 participants, 1 RCT with quality score ≥ 7), UDCA for 1.5 to 6 months did not significantly improve ALT levels or radiological steatosis⁴⁷⁻⁴⁹ (Fig. 5, Table 1).

Overall, no benefit on metabolic parameters was observed with UDCA. Minor gastrointestinal effects (diarrhea, motility disorders) occurred in up to 45% of subjects on high-dose UDCA.

Antioxidants

The proposed pathogenetic role of oxidative stress in NAFLD/NASH prompted evaluation of different antioxidants, including vitamins C and E, methyl donors (betaine) aiming at restoring reduced hepatic glutathione stores, silymarin and silybin, free radical scavengers with antifibrotic activity, and Viucid, a nutritional supplement with different antioxidant molecules.

Trials in NASH. Eight RCTs (508 participants, quality score ranging 3-9) evaluated antioxidants in NASH, with overall no significant benefit on liver enzymes^{5,45,50-54} (Fig. 5; Supporting Figs. 1, 2).

Pooled results of the five HQ RCTs (quality score ≥ 8) showed no histological benefit with antioxidants. However, heterogeneity of these studies was high with respect to type and dose of drug, population (pediatric versus adult), treatment duration, and addition of lifestyle intervention. Heterogeneity was entirely explained by the two placebo-controlled RCTs with positive results (Figs. 2-4): in the Dufour study, 2 years of UDCA + vitamin E significantly improved histological

steatosis compared with double placebo. In the Pioglitazone Versus Vitamin E Versus Placebo for the Treatment of Nondiabetic Patients with NASH trial,²³ vitamin E improved steatosis, ballooning, and inflammation (Figs. 2-4) and reversed NASH in half of the patients versus 25% patients on placebo. The primary endpoint was reached by 43% of patients with vitamin E versus 34% of patients on pioglitazone and 19% of patients on placebo ($P = 0.001$ vitamin E versus placebo; $P = 0.23$ vitamin E versus pioglitazone). In these RCTs, no significant change in body weight or insulin sensitivity occurred with antioxidants.

Trials in NAFLD. Four RCTs (362 participants, one RCT with quality score ≥ 7) assessed antioxidants in NAFLD.^{27,49,55,56} Pooled analysis of RCTs showed significant ALT improvement with vitamin E; betaine and silybin significantly improved ultrasonographic steatosis as well (Fig. 5). In the HQ RCT assessing Viucid plus lifestyle intervention,⁵⁶ both arms lost 11% body weight and improved steatosis and NAS compared with baseline, but histological improvement was more consistent with Viucid. No significant metabolic effect or adverse event was observed with antioxidants. No publication bias was detected.

The extreme heterogeneity of RCTs prevents any firm conclusion on the effect of antioxidants in NAFLD/NASH. Whether the histological benefit of vitamin E may appear after 2 or more years of treatment^{23,45} or may be enhanced by weight loss⁵⁶ requires confirmation. Long-term safety of vitamin E is also an issue, because doses of 400 IU/day or higher have been associated with an increased all-cause mortality.⁵⁷

Anti-Tumor Necrosis Factor Alpha Agents (Pentoxifylline)

Trials in NASH. In three small placebo-controlled RCTs (75 participants, two with quality score ≥ 7) 3 to 12 months of pentoxifylline significantly improved ALT⁵⁸⁻⁶⁰ (Fig. 5). In the only HQ RCT, 12 months of pentoxifylline significantly improved hepatocyte ballooning and NAS, and decreased hepatic Bip gene expression, an indicator of endoplasmic reticulum stress, compared with placebo (Figs. 2-4). Body mass index, HOMA score, serum adiponectin, and tumor necrosis factor alpha were unchanged in either arm.

Overall, the drug was well-tolerated, without major adverse events.

Two RCTs (clinicaltrials.gov NCT00590161, NCT-00681733) are evaluating pentoxifylline in NASH.

Antihypertensive Drugs

Trials in NASH. An HQ RCT (quality score 7) randomized 54 hypertensive NASH patients to 20 months of valsartan or telmisartan (an angiotensin receptor blockers with PPAR- γ -modulating activity).⁶¹

Both agents improved steatosis; telmisartan significantly improved ballooning, lobular inflammation, and fibrosis compared with valsartan (Figs. 2-4). Telmisartan significantly reduced insulin resistance, plasma triglycerides, and total cholesterol, whereas the blood pressure-lowering effects were similar with either agent. Currently, telmisartan is the only agent that improved fibrosis in NASH: whether the combination of PPAR- γ and angiotensin receptor blockers activity of telmisartan may mediate its extensive metabolic and histological benefits awaits confirmation in larger RCTs.

Endocannabinoid Receptor Antagonists

Trials in NAFLD. The effect of rimonabant on computed tomography-assessed steatosis was assessed in abdominally obese, dyslipidemic subjects with NAFLD from the ADAGIO-Lipids trial (quality score 7).⁶² Rimonabant reversed computed tomography-assessed steatosis in 48% of patients versus 19% on placebo ($P = 0.03$) and significantly decreased aminotransferases, abdominal/visceral/subcutaneous fat, blood pressure, plasma lipoproteins, and C-reactive protein. Although patients with a history of depression were excluded, adverse effects led to discontinuation of the drug in 13.8% of cases and included gastrointestinal, depressive (2.0% versus 1.3% of placebo), and anxiety disorders (2.2% versus 1.0%). Whereas the benefit of rimonabant on cardiometabolic profile and hepatic steatosis emerged, its safety and histological benefit in NASH are unknown. Concern about depression, anxiety, and suicidal risk led the Food and Drug Administration to deny drug approval in the United States.

L-Carnitine

Trials in NASH. In an HQ RCT (quality score 9), L-carnitine, a modulator of mitochondrial FFA transport and oxidation, improved steatosis, NAS, and aminotransferases when added to lifestyle intervention for 6 months (Figs. 2-5).

L-carnitine was well tolerated and also improved also HOMA, plasma glucose, and total and low-density lipoprotein-cholesterol compared with placebo.⁶³

Bariatric Surgery

The rationale, efficacy, and safety of bariatric surgery (BS) in 15 studies (766 paired liver biopsies) have

been systematically reviewed.⁶⁴ Overall, BS was safe, steatosis resolved in 91.6% (95% CI: 82.4%-97.5%), steatohepatitis improved in 81.3% (61.9%-94.9%), fibrosis in 65.5% (38.2%-88.1%) of cases; NASH resolved in 69.5% (42.4%-90.8%) of cases. However, most studies were retrospective or observational, with different lengths of follow-up, and some showed limited or no improvement in moderate to advanced fibrosis.⁶⁵ In a recent 5-year prospective study on 381 subjects without advanced liver disease, although steatosis, ballooning, and NAS improved significantly and NASH resolved in 48% of cases, fibrosis worsened slightly but significantly (fibrosis score was 1 or less in more than 95% of patients after BS).⁶⁶ Most of the improvement occurred within 1 year, and the persistence of insulin resistance at 1 year, rather than the degree of weight loss, predicted lack of histological response at 5 years.⁶⁶ Therefore, although promising, long-term effects of BS warrant prospective assessment in homogeneous well-designed trials.

Discussion

Our analysis highlights the limitations of available evidence for the treatment of NAFLD. Fifty-three percent of RCTs assessed biochemical or radiological steatosis, lacking post-treatment histology. Liver enzymes and even steatosis spontaneously fluctuate over time in NAFLD, and their improvement may simply reflect "regression to the mean" rather than treatment efficacy, especially when patients are selected on the basis of elevated liver enzymes. Furthermore, aminotransferases and hepatic steatosis often do not parallel the course of necroinflammation and fibrosis in NASH, improving over time whereas necroinflammation and fibrosis remain stable or progress.^{14-16,44,46} Only 21% of negative RCTs reported *a priori* sample size calculation, which limits their negative predictive power. Finally, the short duration of trials, not exceeding 2 years, prevents any conclusion on long-term efficacy and safety of proposed treatments; neither do they let us know whether the observed histological and metabolic improvement will translate into a clinical benefit in terms of liver-related and cardiometabolic morbidity and mortality.

Given these premises, it is clear that available data represent clues for future research and trial design, rather than providing the basis for evidence-based clinical recommendations.

Available RCTs suggest that weight loss is safe and may dose-dependently improve histological disease activity and associated cardiometabolic risk factors in NASH: a 5% weight loss improved steatosis and associate

metabolic parameters, but higher degrees of weight loss were required to ameliorate necroinflammation and overall disease activity. Future trials need to confirm these dose-dependent effects and to assess whether an upper threshold weight loss exists beyond which little histological improvement occurs. A gradual weight loss (in other words, <1.6 kg/week) would also be advisable, because faster weight loss has exacerbated liver injury.⁶⁷ Long-term durability of achieved benefits and patient adherence to weight-losing regimens are also a concern, because only approximately 40% of patients achieved target weight loss, even in those trials implementing multidisciplinary lifestyle interventions and behavioral therapy.⁴

Increasing evidence also suggests regular physical activity *per se* reduces liver fat, independently of its weight-losing effects, and also may protect NAFLD patients against the development of diabetes.⁶⁸

Exercise implementation into lifestyle programs enhanced prolonged weight loss and proved more sustainable over time than dietary intervention alone in NAFLD-associated metabolic disorders,⁶⁹ and the effect of physical activity on liver histology in NASH warrant further evaluation. Another major field of research regards the optimal dietary nutrient composition for NASH, for which we found only one RCT without histological data.

For patients with NASH unable to achieve or maintain lifestyle-induced weight loss, pharmacological treatment could be considered. Currently, no specific pharmacological treatment outside clinical trials can be recommended, for the limitations discussed. TZDs (mainly pioglitazone) and antioxidants have been most extensively evaluated in HQ RCTs: whereas TZDs consistently improved steatosis and inflammation, RCTs with antioxidants were extremely heterogeneous and yielded conflicting results, showing histological benefit over 2 years or when implemented with weight-losing regimens.^{23,45,56} However, long-term efficacy and safety of TZDs are unknown, and not all patients respond to TZDs. Only a few RCTs^{16,18} evaluated predictors of response to pharmacological treatment: future trials should address these issues, individuating clinical predictors of response, to individualize therapy to the patient's clinical characteristics. The results of recent RCTs suggest that therapeutic strategies other than insulin sensitizers also may be effective in NASH^{23,60,61} and that a combination therapy targeting multiple mechanisms involved in the pathogenesis of NASH may be required. Future trials need to assess, in particular, whether implementing effective weight-loss regimens with drugs has synergistic benefits on liver histology.^{56,63}

With the exception of telmisartan,⁶¹ available treatments show no consistent benefit on hepatic fibrosis; this may be attributable to an actual ineffectiveness of proposed treatments, to a short trial duration, or to the inclusion of subjects with mild degrees of fibrosis. Longer follow-up will tell whether improvement of inflammatory changes may favorably affect clinical outcomes, because inflammation at initial biopsy independently predicted fibrosis progression in NASH over 5 years.⁷⁰ Clearly, future RCTs need to have histological endpoints, to have adequate power and duration, and to enroll patients with the whole spectrum of fibrosis severity; a longer duration will also allow the assessment of long-term safety, durability, and benefits of proposed treatments on patient-oriented outcomes, including liver-related (for example, cirrhosis, liver failure, hepatocellular carcinoma), cardiovascular, and metabolic morbidity, which all contribute to the burden of NAFLD.

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 Roberto Gambino: data collection and elaboration
 Maurizio Cassader: data collection and elaboration
 Gianfranco Pagano: data collection and elaboration

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