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SYSTEMATIC REVIEW

Impact of current treatments on liver disease, glucose metabolism and cardiovascular risk in non-alcoholic fatty liver disease (NAFLD): a systematic review and meta-analysis of randomised trials

G. Musso · M. Cassader · F. Rosina · R. Gambino

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Abstract
Aims/hypothesis Non-alcoholic fatty liver disease (NAFLD) encompasses a spectrum ranging from simple steatosis to non-alcoholic steatohepatitis (NASH): NAFLD causes an increased risk of cardiovascular disease, diabetes and liver-related complications (the latter confined to NASH). The effect of proposed treatments on liver disease, glucose metabolism and cardiovascular risk in NAFLD is unknown. We reviewed the evidence for the management of liver disease and cardio-metabolic risk in NAFLD.
Methods Publications through November 2011 were systematically reviewed by two authors. Outcomes evaluated though standard methods were: histological/radiological/biochemical features of NAFLD, variables of glucose metabolism and cardiovascular risk factors. Seventy-eight randomised trials were included (38 in NASH, 40 in NAFLD): 41% assessed post-treatment histology, 71% assessed glucose metabolism and 88% assessed cardiovascular risk factors. Lifestyle intervention, thiazolidinediones, metformin and antioxidants were most extensively evaluated.
Results Lifestyle-induced weight loss was safe and improved cardio-metabolic risk profile; a weight loss ≥7% improved histological disease activity, but was achieved by <50% patients. Statins and polyunsaturated fatty acids improved steatosis, but their effects on liver histology are unknown. Thiazolidinediones improved histological disease activity, glucose, lipid and inflammatory variables and delayed fibrosis progression. Pioglitazone also improved blood pressure. Weight gain (up to 4.8%) was common. Antioxidants yielded mixed histological results: vitamin E improved histological disease activity when administered for 2 years, but increased insulin resistance and plasma triacylglycerols.
Conclusions/interpretation Weight loss is safe, and improves liver histology and cardio-metabolic profile. For patients not responding to lifestyle intervention, pioglitazone improves histological disease activity, slows fibrosis progression and extensively ameliorates cardio-metabolic endpoints. Further randomised controlled trials (RCTs) of adequate size and duration will assess long-term safety and efficacy of proposed treatments on clinical outcomes.

Keywords Fatty liver · Human · Management · Meta-analysis · NAFLD · NASH · Systematic review

Abbreviations

ALT	Alanine aminotransferase
CB1	Cannabinoid type 1 receptor
CRP	C-reactive protein
CT	Computed tomography
CVD	Cardiovascular disease
EOT	End of treatment
FPG	Fasting plasma glucose
FLIRT	Fatty Liver Improvement with Rosiglitazone Therapy
FXR	Farnesoid X receptor
GLP-1	Glucagon-like peptide-1
GREACE	Greek Atorvastatin and Coronary Heart Disease Evaluation

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MRS	Magnetic resonance spectroscopy
NAFLD	Non-alcoholic fatty liver disease
NAS	NAFLD activity score
NASH	Non-alcoholic steatohepatitis
NMR	Nuclear magnetic resonance
PGC1α	Peroxisome proliferator activated receptor-γ coactivator 1α
PIVENS	Pioglitazone Versus Vitamin E Versus Placebo for the Treatment of Non-diabetic Patients with NASH
PPAR	Peroxisome proliferator activated receptor
PUFA	Polyunsaturated fatty acid
RCT	Randomised controlled trial
TG	Triacylglycerol
UDCA	Ursodeoxycholic acid
WMD	Weighed mean difference

Introduction

Non-alcoholic fatty liver disease (NAFLD) affects 30% of the general adult population and 60–80% of diabetic and obese patients [1, 2]. NAFLD encompasses a histological spectrum ranging from simple steatosis (SS) to steatosis plus necro-inflammation (non-alcoholic steatohepatitis, NASH), with or without fibrosis, that can only be differentiated by liver biopsy. NAFLD carries an increased risk of (1) liver-related complications: whereas SS is considered to have a benign hepatological prognosis, NASH progresses to cirrhosis in 20–25% of cases over 10 years [1]; (2) cardio-metabolic complications: NAFLD confers an increased risk of cardiovascular disease (CVD) and diabetes [3] both directly and through its association with other cardio-metabolic abnormalities, including obesity and metabolic syndrome [4]. Therefore, the impact of proposed treatments on cardio-metabolic profile, as well as on liver disease, should be evaluated. We systematically reviewed the effect of current non-surgical treatments on liver disease and cardio-metabolic risk in NAFLD.

Methods

Data sources and study selection

A detailed description of data sources and searches, and of study selection, is reported in the electronic supplementary material (ESM).

Outcome measures

Liver disease Primary outcome measures were incident cirrhosis/liver failure/hepatocellular carcinoma and improvement in hepatic histological features (steatosis, hepatocellular ballooning, lobular inflammation, fibrosis and, when separate histological features were unavailable, NAFLD activity score, NAS, which is the sum of steatosis, hepatocellular ballooning and lobular inflammation); wherever possible, the impact on fibrosis progression (i.e. the number of patients with unchanged or improved fibrosis stage) was also assessed. When these outcomes were unavailable, changes in radiological steatosis (by ultrasonography, nuclear magnetic resonance [NMR] magnetic resonance spectroscopy [MRS] or computed tomography [CT]), and in serum alanine aminotransferase (ALT) were evaluated.

Glucose metabolism We evaluated incident diabetes, fasting plasma glucose (FPG), glucose tolerance (as assessed by a standard OGTT), HbA_{1c}, HOMA index and other variables related to insulin sensitivity (hepatic and extrahepatic) and insulin secretion, BMI and abdominal obesity (assessed by anthropometry or by NMR/CT).

Cardiovascular risk We evaluated incident cardiovascular events, BP, plasma lipids (triacylglycerol, LDL- and HDL-cholesterol) and inflammatory markers/cytokines, including C-reactive protein (CRP), adiponectin, interleukin-6 and TNF-α.

Incident adverse events were also evaluated.

The quality of randomised controlled trials (RCTs) was assessed by the Cochrane Risk of Bias Tool (score range: 0–8) [5]. RCTs scoring >6 were arbitrarily considered as having a low bias risk.

Results

The agreement for study selection between the two reviewers was good (κ coefficient=0.86). We retrieved 78 RCTs (47 with a low risk of bias), variably reporting post-treatment changes in liver-related, glucose and cardiovascular variables (Table 1; ESM Fig. 1; ESM Tables 1-5).

Weight loss

Eight RCTs (373 participants, 39% diabetic; six RCTs with a low risk of bias, four RCTs with post-treatment histology) assessed the effect of lifestyle- or drug-induced weight loss in NAFLD [6–13] (ESM Table 1).

Liver disease Although a ≥5% weight loss improved hepatic steatosis, a ≥7% weight loss also improved NAS (Fig. 1); fibrosis was unchanged (not shown). The threshold of 7% weight loss was achieved by <50% of patients, even with intensive multidisciplinary lifestyle intervention [8, 10]. Two

Table 1 Items related to liver disease, glucose metabolism and cardiovascular risk and the percentage of RCTs assessing their post-treatment changes (total: 78 RCTs included)

Item assessed	Method	RCTs with post-treatment changes (%)
Liver disease		
Liver histology	Liver biopsy	41
Radiological steatosis		45
	Ultrasound	17
	MRI	21
	CT	8
Liver enzymes	AST, ALT, GGT	93
Adiposity		
Whole body adiposity	BMI	99
Abdominal adiposity		37
	Waist	24
	Waist-on-hip ratio	4
	MRI	9
	CT	4
Glucose homeostasis		
Pancreatic beta cell function	OGTT-derived indices of pancreatic beta cell function	3
Insulin sensitivity		71
	Fasting indices (HOMA, QUICKI)	55
	OGTT-derived indices	8
	FSIVGTT	1
	Hyperinsulinemic euglycaemic glucose clamp-derived indices	9
Plasma glucose control		
FPG		76
Glucose tolerance	2 h plasma glucose on OGTT	17
HbA_{1c}	–	22
Plasma lipids		
Fasting plasma triacylglycerols, total cholesterol/LDL-cholesterol/ HDL-cholesterol		79
BP		
Systolic/diastolic BP		22
Chronic systemic inflammation		
Pro-/anti-inflammatory cytokines		40
	Adiponectin	24
	C-reactive protein	19
	TNF-α	8
	Interleukin-6	4
	TGF-β, FGF-18, ICAM-1, VCAM-1	2

FGF, fibroblast growth factor; FSIVGTT, frequently sampled intravenous glucose tolerance test; ICAM, intercellular adhesion molecule; TGF, transforming growth factor; VCAM, vascular cellular adhesion molecule

RCTs suggested no additional NAS improvement with >10% weight loss, but the existence of a lower and an upper threshold weight loss for improving histological disease activity needs further confirmation (Fig. 2).

There was no significant publication bias (ESM Fig. 2).

Glucose metabolism and cardiovascular risk Weight loss substantially improved HOMA, FPG, glucose tolerance and plasma lipids (ESM Table 1). Two RCTs also showed an improvement in plasma adiponectin [8, 12]. Among drugs inducing weight loss, orlistat was safe, well-tolerated with minor adverse gastrointestinal complaints not requiring discontinuation of therapy, but conferred no additional cardio-metabolic or histological benefit over lifestyle intervention alone [7, 12]. There was no significant publication bias for assessed outcomes (not reported).

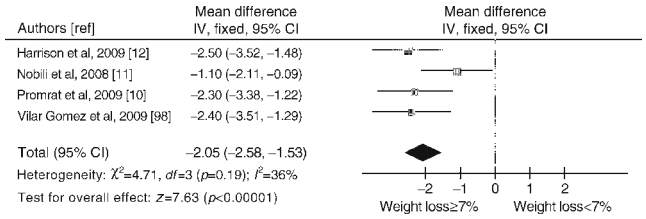


Fig. 1 Forest plot of RCTs comparing the effect of different degrees of weight loss (%) on histological NAS. Outcome: mean differences in NAS following weight loss ≥7% vs weight loss <7%. IV, inverse variance

Long-term durability of achieved benefits and safety of weight loss are unknown.

Physical exercise alone

Reduced aerobic exercise has been linked to the presence and severity of cardio-metabolic and liver disease in NAFLD through several potential mechanisms: reduced hepatic and muscle adenosine monophosphate-activated protein kinase (AMPK)-mediated NEFA oxidation, increased postprandial hepatic lipogenesis, visceral fat-derived NEFA and proinflammatory adipokine overflow to the liver [14–17].

Five RCTs (four RCTs with a low risk of bias) evaluated the effects of 3–6 months of moderate-intensity aerobic exercise alone in NAFLD [13, 18–21] (ESM Table 1).

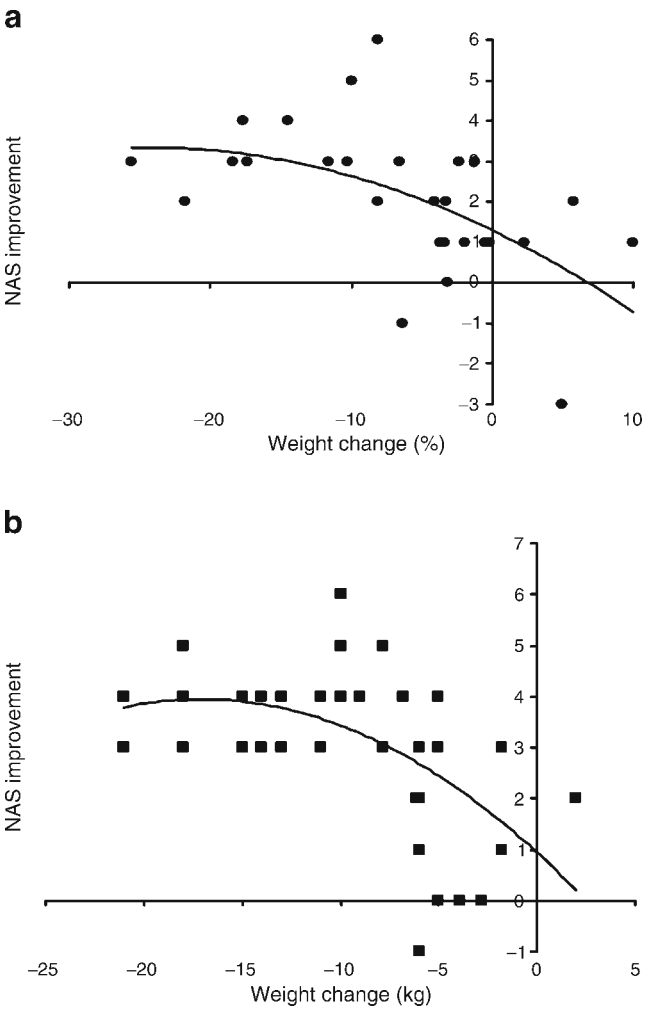


Fig. 2 Impact of different degrees of weight loss on histological NAS in two RCTs (adapted from (**a**) Promrat et al [10] and (**b**) Vilar Gomez et al [98])

Liver disease Exercise improved MRS-assessed steatosis and ALT levels (Fig. 3). In the only RCT with post-treatment histology, NAS was unchanged [13]. There was no significant publication bias (ESM Fig. 2)

Glucose metabolism and cardiovascular risk Despite no significant body weight changes, exercise improved waist circumference, HOMA, FPG, HbA_{1c}, LDL-cholesterol and triacylglycerol (TG) (Fig. 3). One RCT reported no effect of physical exercise on HDL-cholesterol [20]. No data on inflammatory markers/adipokines are available. There was no significant publication bias for assessed outcomes (not reported).

An analysis of the reasons for dropping out of exercise-based treatments found that NAFLD patients understand the benefits of exercise but lack confidence to perform it, and are afraid of falling, suggesting that these modifiable factors should be targeted to improve compliance to exercise of these patients [22].

Dietary composition manipulation

The optimal nutrient dietary composition for NAFLD is unknown. Three RCTs compared the effect of low-carbohydrate versus low-fat caloric restriction [23–25] (ESM Table 1).

Liver disease The two regimens yielded similar liver fat and ALT reduction (Fig. 4).

Glucose metabolism and cardiovascular risk The two regimens yielded similar weight loss and improved HOMA, pancreatic beta cell function [24], TG, blood pressure [25], CRP [24] and adiponectin to a similar extent (Fig. 4). For TG and HOMA heterogeneity was high, being explained by the different baseline features of study populations: low-carbohydrate diet significantly improved plasma TG and HOMA index when hypertriacylglycerolaemic [25] or glucose-intolerant [23] NAFLD patients, respectively, were enrolled. Furthermore, in glucose-intolerant NAFLD individuals, low-carbohydrate caloric restriction significantly improved hepatic insulin sensitivity compared with low-fat diet [23].

Low-carbohydrate diet significantly reduced waist circumference and FPG compared with low-fat diet, which in

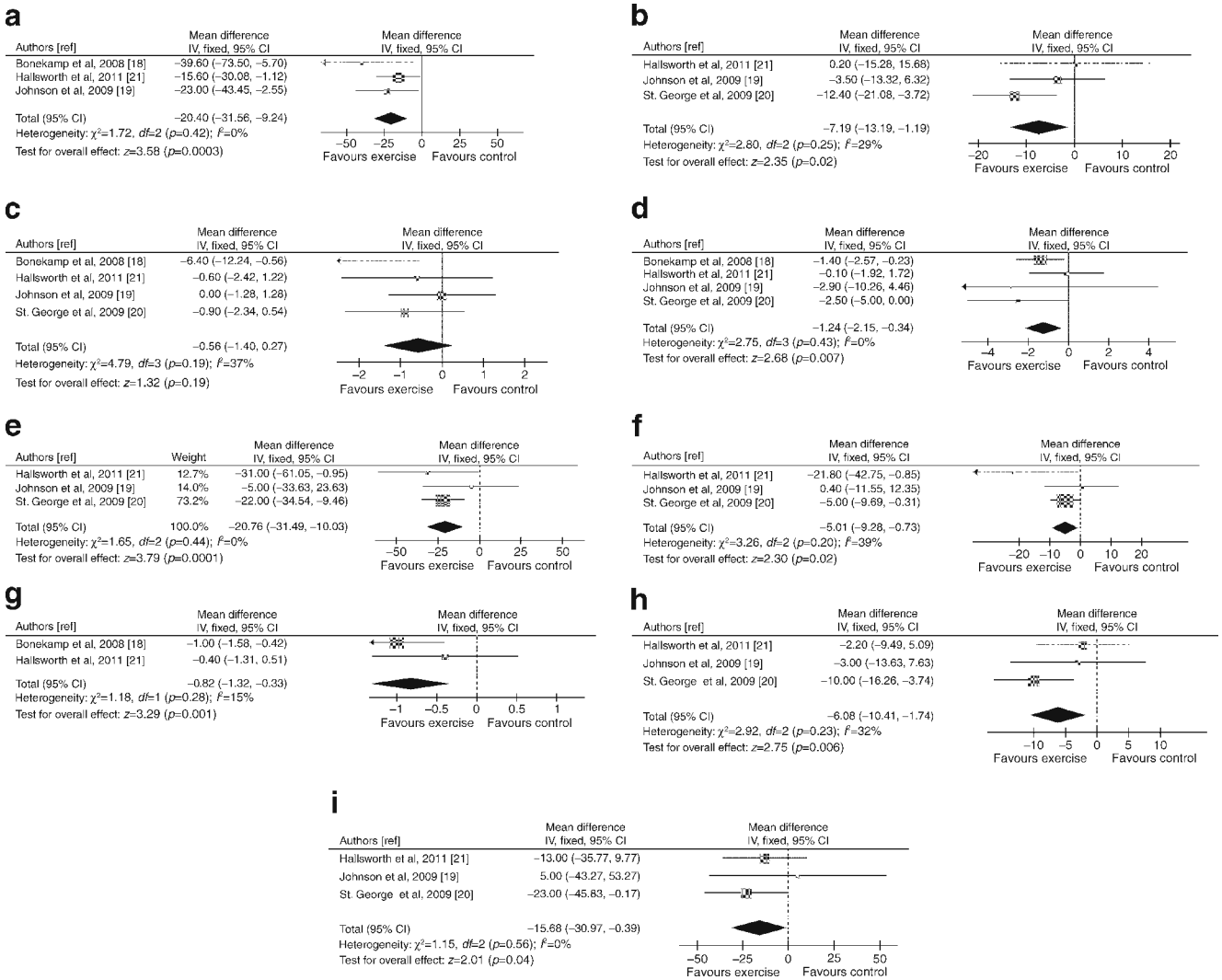


Fig. 3 Forest plots of RCTs comparing the effect of physical exercise alone on liver disease, glucose metabolism and cardiovascular risk. (**a**) NMR-assessed liver fat change (%). (**b**) ALT change (IU/l). (**c**) Body weight change (%). (**d**) Waist circumference change (%). (**e**) HOMA index change (%). (**f**) FPG change (%). (**g**) HbA_{1c} change (%). (**h**) Plasma LDL-cholesterol change (%). (**i**) Plasma TG change (%).To convert values for HbA_{1c} in % into mmol/mol, subtract 2.15 and multiply by 10.929. IV, inverse variance

turn improved LDL-C and HDL-C more consistently than the low-carbohydrate diet (Fig. 4).

These studies suggest that caloric restriction is the most important goal for improving hepatic steatosis, but a different nutrient composition may carry additional benefits according to individual patient features.

Insulin-sensitisers: thiazolidinediones

Thiazolidinediones (TZDs) were evaluated in 11 RCTs (862 participants, 38% diabetic; seven RCTs with low risk of bias) [26–37] (ESM Table 2).

Liver disease Pooled results of seven RCTs with post-treatment histology showed that TZDs improved steatosis, hepatocellular ballooning and inflammation but not fibrosis; however, when considering patients with improved or stable fibrosis stage versus those with worsening fibrosis stage, TZDs significantly reduced the risk of fibrosis progression (Fig. 5). Heterogeneity was low for all assessed outcomes, suggesting a consistent drug effect size across studies. There was no significant publication bias (ESM Fig. 2)

Presence/absence of diabetes, the implementation of life-style intervention, different drug, dose or trial duration and risk of bias did not affect outcomes.

Glucose metabolism and cardiovascular risk TZDs improved HOMA, FPG, HbA_{1c}, HDL-C, TG, CRP and adiponectin, but had no effect on LDL-C and BP (Fig. 5). TZDs improved also hepatic, muscle and adipose tissue insulin resistance [26, 34, 37]. There was no significant publication bias for assessed outcomes (not reported).

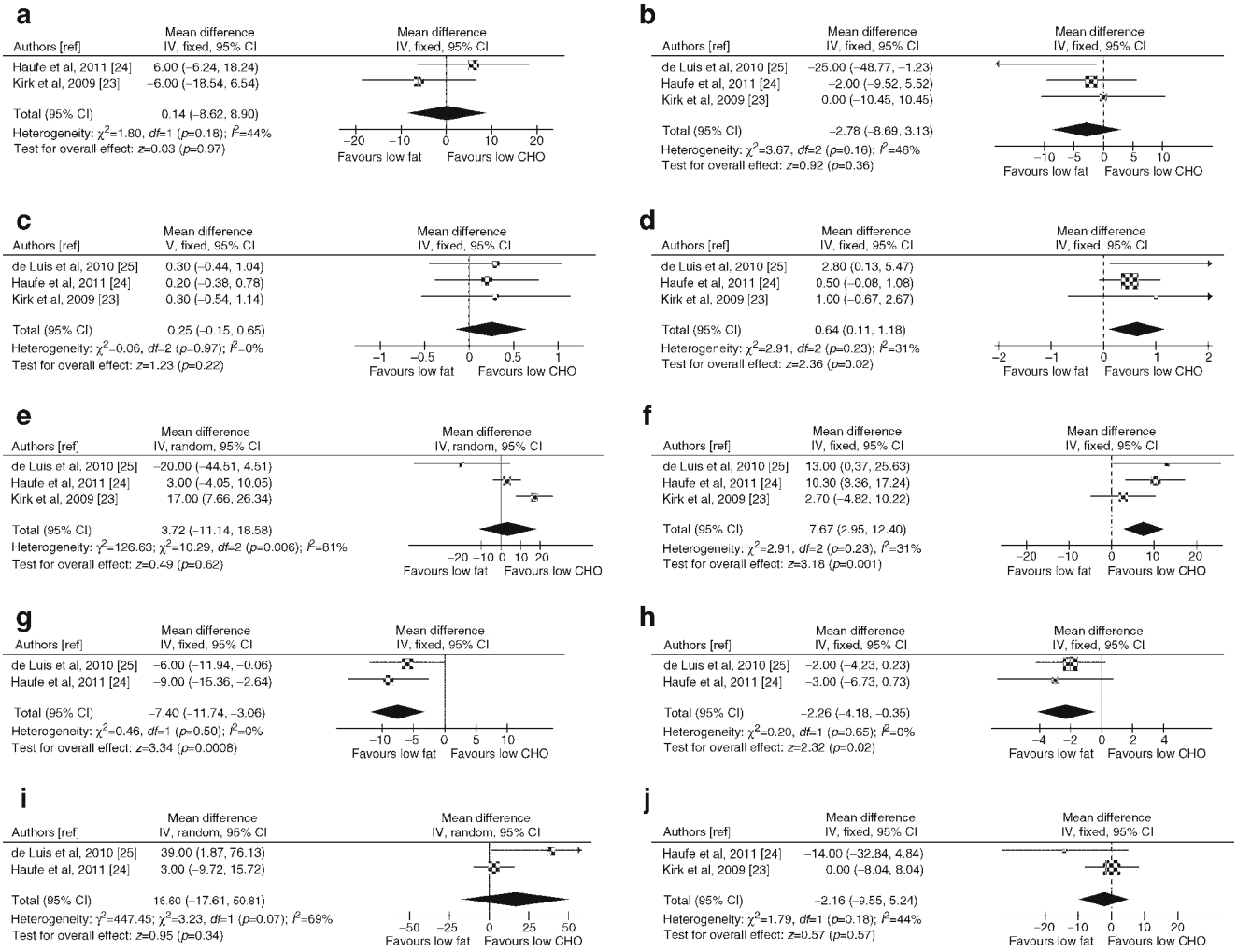


Fig. 4 Forest plots of RCTs comparing the effect of low fat versus low carbohydrate (CHO) dietary caloric restriction on liver disease, glucose metabolism and cardiovascular risk. (**a**) NMR-assessed liver fat change (%). (**b**) ALT change (IU/l). (**c**) Body weight change (%). (**d**) Waist circumference change (%). (**e**) HOMA index change (%). (**f**) FPG change (%). (**g**) Plasma LDL-cholesterol change (%). (**h**) Plasma HDL-cholesterol change (%) (**i**) Plasma TG change (%). (**j**) Serum adiponectin change (%). IV, inverse variance

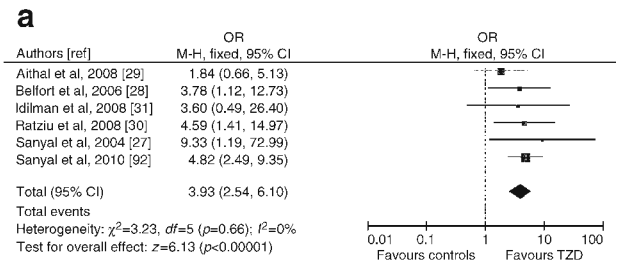
For some outcomes heterogeneity was high: for LDL-C, heterogeneity was abated after excluding one RCT [30], showing unexpected LDL-C increase with rosiglitazone (weighed mean difference [WMD] 1.13, 95% CI −2.40, 4.66, p=0.53, I^2=34%, n comparisons=5). For HOMA, heterogeneity was abated after excluding one RCT [29], showing unexpected HOMA increase with pioglitazone (WMD −33%, 95% CI −44%, −22%, p=0.00001, I^2=40%, n comparisons=7).

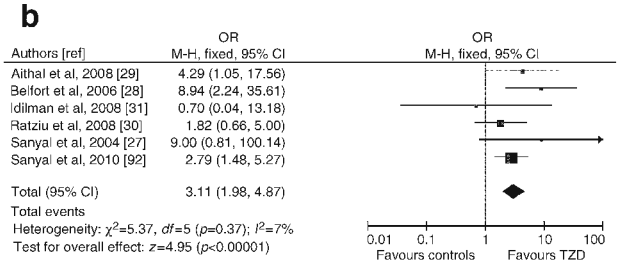
For BP, after excluding the only RCT using rosiglitazone [34], the remaining trials showed no change in systolic BP (WMD −1.5%, 95% CI −4.4%, −1.2%, p=0.27, I^2=12%, n comparisons=3) or a reduction in diastolic BP (WMD −3.3%, 95% CI −5.5%, −1.0%, p=0.005, I^2=0%, n comparisons=3) with pioglitazone.

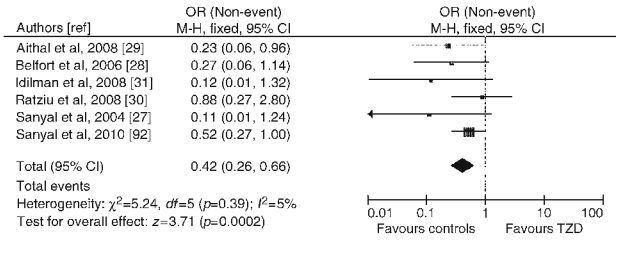
For adiponectin, heterogeneity was abated after excluding two RCTs using a lower dose of pioglitazone [29] or did not vigorously implement lifestyle intervention [30] (WMD 118%, 95% CI 82, 155, p=0.00001, I^2=0%, n comparisons=3).

Weight gain (mean 2%, range 0–4.8%) occurred in up to 75% of patients, accompanied by an increased in waist circumference, and was a common cause of dropout, together

Fig. 5 Forest plots of RCTs comparing the effect of thiazolidinedione on liver disease, glucose metabolism and cardiovascular risk. (**a**) Improvement in histological steatosis in NASH. (**b**) Improvement in lobular inflammation in NASH. (**c**) Improvement in hepatocellular ballooning in NASH. (**d**) Improvement in fibrosis in NASH. (**e**) Improvement or stability in fibrosis in NASH. (**f**) Body weight change (%). (**g**) Waist circumference change (%). (**h**) Systolic BP changes (mmHg). (**i**) Diastolic BP changes (mmHg). (**j**) HOMA index change (%). (**k**) FPG change (%). (**l**) HbA_{1c} change (%). (**m**) Plasma LDL-cholesterol change (%). (**n**) Plasma HDL-cholesterol change (%) (**o**) Plasma TG change (%). (**p**) Serum C-reactive protein change (mg/l). (**q**) Serum adiponectin change (%). To convert values for HbA_{1c} in % into mmol/mol, subtract 2.15 and multiply by 10.929. M-H, Mantel–Haenszel


a
Authors [ref]
OR M-H, fixed, 95% CI
Aithal et al, 2008 [29] 1.84 (0.66, 5.13)
Belfort et al, 2006 [28] 3.78 (1.12, 12.73)
Idilman et al, 2008 [31] 3.60 (0.49, 26.40)
Ratziu et al, 2008 [30] 4.59 (1.41, 14.97)
Sanyal et al, 2004 [27] 9.33 (1.19, 72.99)
Sanyal et al, 2010 [92] 4.82 (2.49, 9.35)
Total (95% CI) 3.93 (2.54, 6.10)
Total events
Heterogeneity: χ²=3.23, df=5 (p=0.66); I²=0%
Test for overall effect: z=6.13 (p<0.00001)
0.01 0.1 1 10 100
Favours controls Favours TZD


b
Authors [ref]
OR M-H, fixed, 95% CI
Aithal et al, 2008 [29] 4.29 (1.05, 17.56)
Belfort et al, 2006 [28] 8.94 (2.24, 35.61)
Idilman et al, 2008 [31] 0.70 (0.04, 13.18)
Ratziu et al, 2008 [30] 1.82 (0.66, 5.00)
Sanyal et al, 2004 [27] 9.00 (0.81, 100.14)
Sanyal et al, 2010 [92] 2.79 (1.48, 5.27)
Total (95% CI) 3.11 (1.98, 4.87)
Total events
Heterogeneity: χ²=5.37, df=5 (p=0.37); I²=7%
Test for overall effect: z=4.95 (p<0.00001)
0.01 0.1 1 10 100
Favours controls Favours TZD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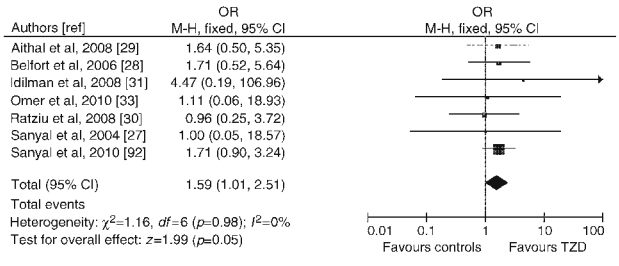
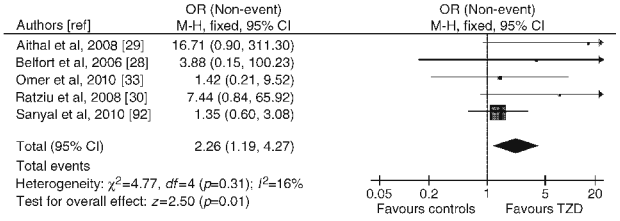
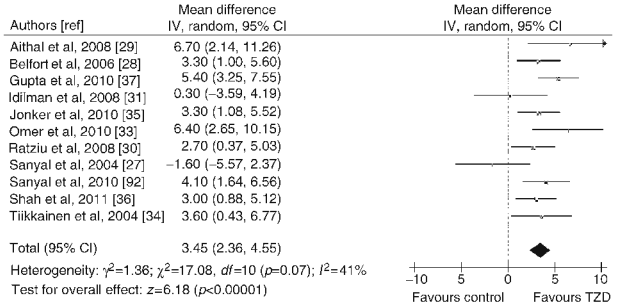
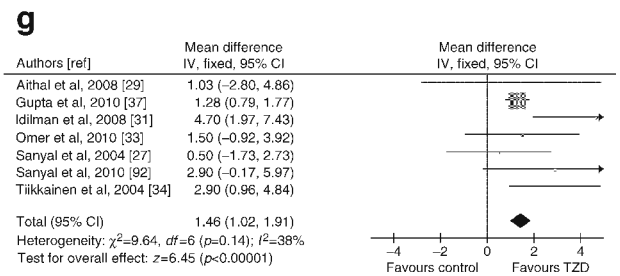
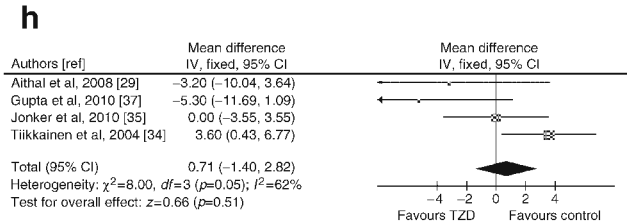
Authors [ref]
OR (Non-event) M-H, fixed, 95% CI
Aithal et al, 2008 [29] 0.23 (0.06, 0.96)
Belfort et al, 2006 [28] 0.27 (0.06, 1.14)
Idilman et al, 2008 [31] 0.12 (0.01, 1.32)
Ratziu et al, 2008 [30] 0.88 (0.27, 2.80)
Sanyal et al, 2004 [27] 0.11 (0.01, 1.24)
Sanyal et al, 2010 [92] 0.52 (0.27, 1.00)
Total (95% CI) 0.42 (0.26, 0.66)
Total events
Heterogeneity: χ²=5.24, df=5 (p=0.39); I²=5%
Test for overall effect: z=3.71 (p=0.0002)
0.01 0.1 1 10 100
Favours controls Favours TZD

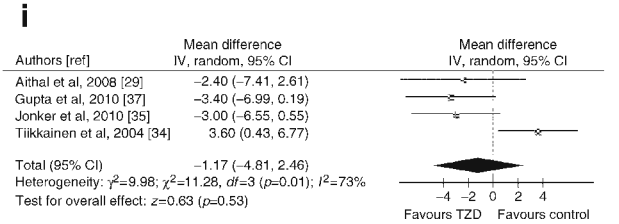

d
Authors [ref]
OR M-H, fixed, 95% CI
Aithal et al, 2008 [29] 1.64 (0.50, 5.35)
Belfort et al, 2006 [28] 1.71 (0.52, 5.64)
Idilman et al, 2008 [31] 4.47 (0.19, 106.96)
Omer et al, 2010 [33] 1.11 (0.06, 18.93)
Ratziu et al, 2008 [30] 0.96 (0.25, 3.72)
Sanyal et al, 2004 [27] 1.00 (0.05, 18.57)
Sanyal et al, 2010 [92] 1.71 (0.90, 3.24)
Total (95% CI) 1.59 (1.01, 2.51)
Total events
Heterogeneity: χ²=1.16, df=6 (p=0.98); I²=0%
Test for overall effect: z=1.99 (p=0.05)
0.01 0.1 1 10 100
Favours controls Favours TZD

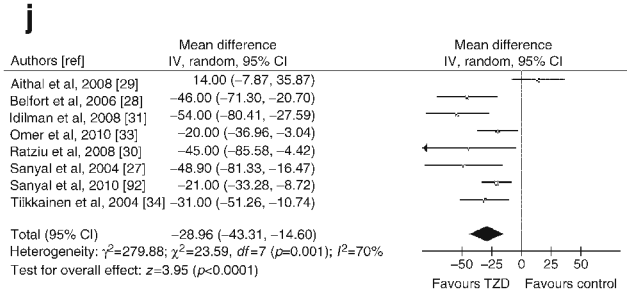

e
Authors [ref]
OR (Non-event) M-H, fixed, 95% CI
Aithal et al, 2008 [29] 16.71 (0.90, 311.30)
Belfort et al, 2006 [28] 3.88 (0.15, 100.23)
Omer et al, 2010 [33] 1.42 (0.21, 9.52)
Ratziu et al, 2008 [30] 7.44 (0.84, 65.92)
Sanyal et al, 2010 [92] 1.35 (0.60, 3.08)
Total (95% CI) 2.26 (1.19, 4.27)
Total events
Heterogeneity: χ²=4.77, df=4 (p=0.31); I²=16%
Test for overall effect: z=2.50 (p=0.01)
0.05 0.2 1 5 20
Favours controls Favours TZD

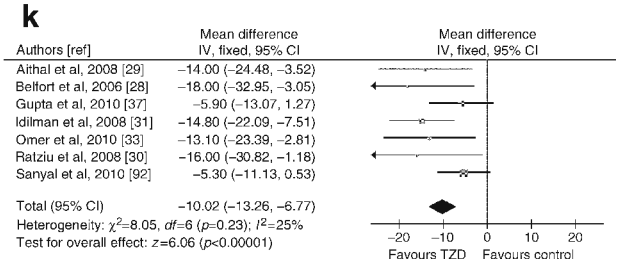

f
Authors [ref]
Mean difference IV, random, 95% CI
Aithal et al, 2008 [29] 6.70 (2.14, 11.26)
Belfort et al, 2006 [28] 3.30 (1.00, 5.60)
Gupta et al, 2010 [37] 5.40 (3.25, 7.55)
Idilman et al, 2008 [31] 0.30 (−3.59, 4.19)
Jonker et al, 2010 [35] 3.30 (1.08, 5.52)
Omer et al, 2010 [33] 6.40 (2.65, 10.15)
Ratziu et al, 2008 [30] 2.70 (0.37, 5.03)
Sanyal et al, 2004 [27] −1.60 (−5.57, 2.37)
Sanyal et al, 2010 [92] 4.10 (1.64, 6.56)
Shah et al, 2011 [36] 3.00 (0.88, 5.12)
Tiikkainen et al, 2004 [34] 3.60 (0.43, 6.77)
Total (95% CI) 3.45 (2.36, 4.55)
Heterogeneity: τ²=1.36; χ²=17.08, df=10 (p=0.07); I²=41%
Test for overall effect: z=6.18 (p<0.00001)
−10 −5 0 5 10
Favours control Favours TZD

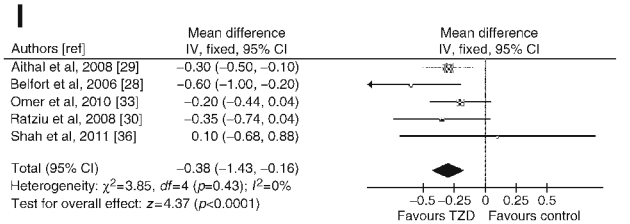

g
Authors [ref]
Mean difference IV, fixed, 95% CI
Aithal et al, 2008 [29] 1.03 (−2.80, 4.86)
Gupta et al, 2010 [37] 1.28 (0.79, 1.77)
Idilman et al, 2008 [31] 4.70 (1.97, 7.43)
Omer et al, 2010 [33] 1.50 (−0.92, 3.92)
Sanyal et al, 2004 [27] 0.50 (−1.73, 2.73)
Sanyal et al, 2010 [92] 2.90 (−0.17, 5.97)
Tiikkainen et al, 2004 [34] 2.90 (0.96, 4.84)
Total (95% CI) 1.46 (1.02, 1.91)
Heterogeneity: χ²=9.64, df=6 (p=0.14); I²=38%
Test for overall effect: z=6.45 (p<0.00001)
−4 −2 0 2 4
Favours control Favours TZD


h
Authors [ref]
Mean difference IV, fixed, 95% CI
Aithal et al, 2008 [29] −3.20 (−10.04, 3.64)
Gupta et al, 2010 [37] −5.30 (−11.69, 1.09)
Jonker et al, 2010 [35] 0.00 (−3.55, 3.55)
Tiikkainen et al, 2004 [34] 3.60 (0.43, 6.77)
Total (95% CI) 0.71 (−1.40, 2.82)
Heterogeneity: χ²=8.00, df=3 (p=0.05); I²=62%
Test for overall effect: z=0.66 (p=0.51)
−4 −2 0 2 4
Favours TZD Favours control


i
Authors [ref]
Mean difference IV, random, 95% CI
Aithal et al, 2008 [29] −2.40 (−7.41, 2.61)
Gupta et al, 2010 [37] −3.40 (−6.99, 0.19)
Jonker et al, 2010 [35] −3.00 (−6.55, 0.55)
Tiikkainen et al, 2004 [34] 3.60 (0.43, 6.77)
Total (95% CI) −1.17 (−4.81, 2.46)
Heterogeneity: τ²=9.98; χ²=11.28, df=3 (p=0.01); I²=73%
Test for overall effect: z=0.63 (p=0.53)
−4 −2 0 2 4
Favours TZD Favours control


j
Authors [ref]
Mean difference IV, random, 95% CI
Aithal et al, 2008 [29] 14.00 (−7.87, 35.87)
Belfort et al, 2006 [28] −46.00 (−71.30, −20.70)
Idilman et al, 2008 [31] −54.00 (−80.41, −27.59)
Omer et al, 2010 [33] −20.00 (−36.96, −3.04)
Ratziu et al, 2008 [30] −45.00 (−85.58, −4.42)
Sanyal et al, 2004 [27] −48.90 (−81.33, −16.47)
Sanyal et al, 2010 [92] −21.00 (−33.28, −8.72)
Tiikkainen et al, 2004 [34] −31.00 (−51.26, −10.74)
Total (95% CI) −28.96 (−43.31, −14.60)
Heterogeneity: τ²=279.88; χ²=23.59, df=7 (p=0.001); I²=70%
Test for overall effect: z=3.95 (p<0.0001)
−50 −25 0 25 50
Favours TZD Favours control


k
Authors [ref]
Mean difference IV, fixed, 95% CI
Aithal et al, 2008 [29] −14.00 (−24.48, −3.52)
Belfort et al, 2006 [28] −18.00 (−32.95, −3.05)
Gupta et al, 2010 [37] −5.90 (−13.07, 1.27)
Idilman et al, 2008 [31] −14.80 (−22.09, −7.51)
Omer et al, 2010 [33] −13.10 (−23.39, −2.81)
Ratziu et al, 2008 [30] −16.00 (−30.82, −1.18)
Sanyal et al, 2010 [92] −5.30 (−11.13, 0.53)
Total (95% CI) −10.02 (−13.26, −6.77)
Heterogeneity: χ²=8.05, df=6 (p=0.23); I²=25%
Test for overall effect: z=6.06 (p<0.00001)
−20 −10 0 10 20
Favours TZD Favours control


l
Authors [ref]
Mean difference IV, fixed, 95% CI
Aithal et al, 2008 [29] −0.30 (−0.50, −0.10)
Belfort et al, 2006 [28] −0.60 (−1.00, −0.20)
Omer et al, 2010 [33] −0.20 (−0.44, 0.04)
Ratziu et al, 2008 [30] −0.35 (−0.74, 0.04)
Shah et al, 2011 [36] 0.10 (−0.68, 0.88)
Total (95% CI) −0.38 (−1.43, −0.16)
Heterogeneity: χ²=3.85, df=4 (p=0.43); I²=0%
Test for overall effect: z=4.37 (p<0.0001)
−0.5 −0.25 0 0.25 0.5
Favours TZD Favours control

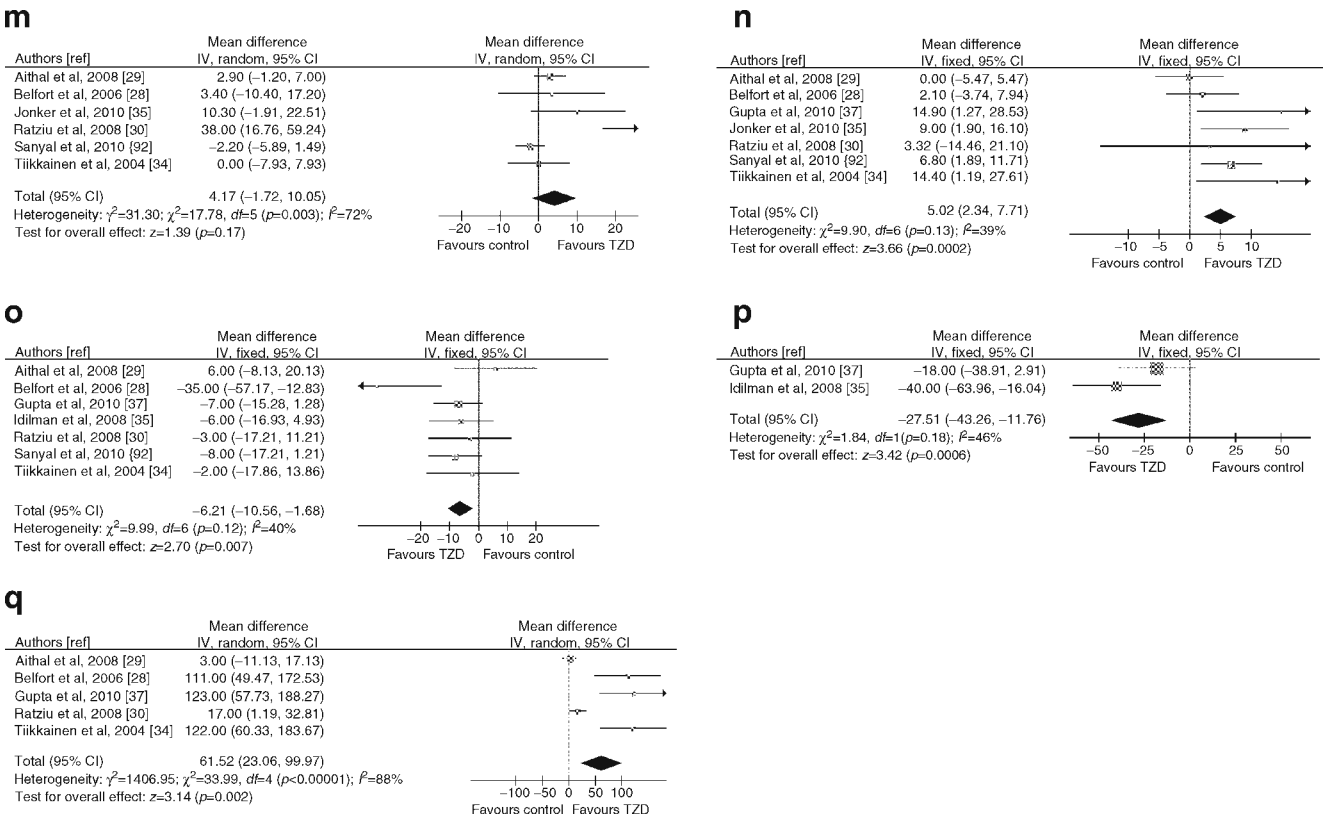


Fig. 5 (continued)

with ankle oedema (4–25%). Weight gain did not reverse with treatment discontinuation and was not prevented by lifestyle intervention, but was reduced by metformin coadministration [33, 38]. Besides limiting weight gain, the combination of rosiglitazone+metformin offered no significant histological or cardio-metabolic benefit over rosiglitazone alone [33, 38].

NASH and associated cardio-metabolic abnormalities relapsed 1 year after discontinuing TZDs [38], posing the issue of the required treatment duration and of the benefit/safety of sustained thiazolidinedione treatment. In the Pioglitazone Versus Vitamin E versus Placebo for the Treatment of Nondiabetic Patients with NASH (PIVENS) and the Fatty Liver Improvement with Rosiglitazone Therapy (FLIRT)-2 trial, liver histology did not improve further despite continued HOMA and transaminase improvement over 2 and 3 years, respectively [32, 39]. These two trials suggest that prolonged treatment with TZDs may offer no additional histological benefit and that metabolic improvement does not necessarily parallel histological improvement.

Due to the short trial duration, no cases of congestive heart failure, bone fractures or CVD events were reported. Concern about cardiovascular safety led the European Medicines Agency to recommend withdrawal of rosiglitazone from clinical use.

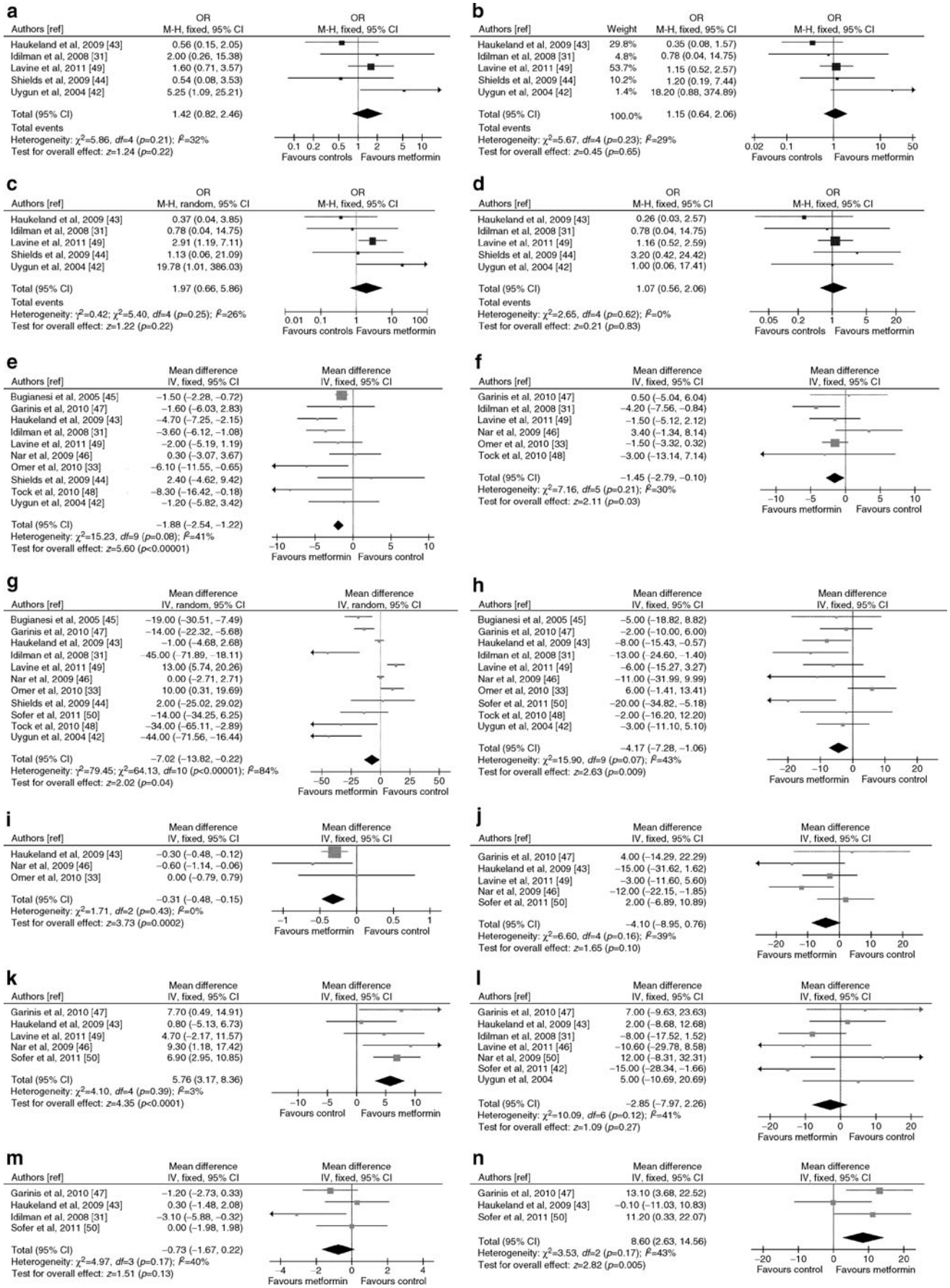
Insulin-sensitisers: metformin

Metformin has anorexigenic and weight-loss properties, decreases gastrointestinal glucose absorption and increases insulin sensitivity by increasing glucose uptake and AMP-kinase-mediated oxidative glucose and lipid metabolism [40].

Eleven RCTs (671 participants, 27% diabetic; six RCTs in NASH with post-treatment histology, three with a low bias risk) evaluated metformin [33, 34, 41–49] (ESM Table 2).

Liver disease Metformin did not improve liver histology compared with placebo (Fig. 6). Dose, treatment duration (ranging from 6 to 24 months) or diabetic state had no effect on post-treatment histology. There was no significant publication bias (ESM Fig. 2)

Fig. 6 Forest plots of RCTs comparing the effect of metformin on liver disease, glucose metabolism and cardiovascular risk. (**a**) Improvement in histological steatosis in NASH. (**b**) Improvement in lobular inflammation in NASH. (**c**) Improvement in hepatocellular ballooning in NASH. (**d**) Improvement in fibrosis in NASH. (**e**) Body weight change (%). (**f**) Waist circumference change (%). (**g**) HOMA index change (%). (**h**) FPG change (%). (**i**) HbA_{1c} change (%). (**j**) Plasma LDL-cholesterol change (%). (**k**) Plasma HDL-cholesterol change (%) (**l**) Plasma TG change (%). (**m**) Serum C-reactive protein change (mg/l). (**n**) Serum adiponectin change (%). To convert values for HbA_{1c} in % into mmol/mol, subtract 2.15 and multiply by 10.929. M-H, Mantel–Haenszel


a
Authors [ref] | OR M-H, fixed, 95% CI
Haukeland et al, 2009 [43] 0.56 (0.15, 2.05)
Idilman et al, 2008 [31] 2.00 (0.26, 15.38)
Lavine et al, 2011 [49] 1.60 (0.71, 3.57)
Shields et al, 2009 [44] 0.54 (0.08, 3.53)
Uygun et al, 2004 [42] 5.25 (1.09, 25.21)
Total (95% CI) 1.42 (0.82, 2.46)
Total events
Heterogeneity: χ²=5.86, df=4 (p=0.21); I²=32%
Test for overall effect: z=1.24 (p=0.22)
0.1 0.2 0.5 1 2 5 10
Favours controls Favours metformin
b
Authors [ref] | Weight | OR M-H, fixed, 95% CI
Haukeland et al, 2009 [43] 29.8% 0.35 (0.08, 1.57)
Idilman et al, 2008 [31] 4.8% 0.78 (0.04, 14.75)
Lavine et al, 2011 [49] 53.7% 1.15 (0.52, 2.57)
Shields et al, 2009 [44] 10.2% 1.20 (0.19, 7.44)
Uygun et al, 2004 [42] 1.4% 18.20 (0.88, 374.89)
Total (95% CI) 100.0% 1.15 (0.64, 2.06)
Total events
Heterogeneity: χ²=5.67, df=4 (p=0.23); I²=29%
Test for overall effect: z=0.45 (p=0.65)
0.02 0.1 1 10 50
Favours controls Favours metformin
c
Authors [ref] | OR M-H, random, 95% CI
Haukeland et al, 2009 [43] 0.37 (0.04, 3.85)
Idilman et al, 2008 [31] 0.78 (0.04, 14.75)
Lavine et al, 2011 [49] 2.91 (1.19, 7.11)
Shields et al, 2009 [44] 1.13 (0.06, 21.09)
Uygun et al, 2004 [42] 19.78 (1.01, 386.03)
Total (95% CI) 1.97 (0.66, 5.86)
Total events
Heterogeneity: τ²=0.42; χ²=5.40, df=4 (p=0.25); I²=26%
Test for overall effect: z=1.22 (p=0.22)
0.01 0.1 1 10 100
Favours controls Favours metformin
d
Authors [ref] | OR M-H, fixed, 95% CI
Haukeland et al, 2009 [43] 0.26 (0.03, 2.57)
Idilman et al, 2008 [31] 0.78 (0.04, 14.75)
Lavine et al, 2011 [49] 1.16 (0.52, 2.59)
Shields et al, 2009 [44] 3.20 (0.42, 24.42)
Uygun et al, 2004 [42] 1.00 (0.06, 17.41)
Total (95% CI) 1.07 (0.56, 2.06)
Total events
Heterogeneity: χ²=2.65, df=4 (p=0.62); I²=0%
Test for overall effect: z=0.21 (p=0.83)
0.05 0.2 1 5 20
Favours controls Favours metformin
e
Authors [ref] | Mean difference IV, fixed, 95% CI
Bugianesi et al, 2005 [45] −1.50 (−2.28, −0.72)
Garinis et al, 2010 [47] −1.60 (−6.03, 2.83)
Haukeland et al, 2009 [43] −4.70 (−7.25, −2.15)
Idilman et al, 2008 [31] −3.60 (−6.12, −1.08)
Lavine et al, 2011 [49] −2.00 (−5.19, 1.19)
Nar et al, 2009 [46] 0.30 (−3.07, 3.67)
Omer et al, 2010 [33] −6.10 (−11.55, −0.65)
Shields et al, 2009 [44] 2.40 (−4.62, 9.42)
Tock et al, 2010 [48] −8.30 (−16.42, −0.18)
Uygun et al, 2004 [42] −1.20 (−5.82, 3.42)
Total (95% CI) −1.88 (−2.54, −1.22)
Heterogeneity: χ²=15.23, df=9 (p=0.08); I²=41%
Test for overall effect: z=5.60 (p<0.00001)
−10 −5 0 5 10
Favours metformin Favours control
f
Authors [ref] | Mean difference IV, fixed, 95% CI
Garinis et al, 2010 [47] 0.50 (−5.04, 6.04)
Idilman et al, 2008 [31] −4.20 (−7.56, −0.84)
Lavine et al, 2011 [49] −1.50 (−5.12, 2.12)
Nar et al, 2009 [46] 3.40 (−1.34, 8.14)
Omer et al, 2010 [33] −1.50 (−3.32, 0.32)
Tock et al, 2010 [48] −3.00 (−13.14, 7.14)
Total (95% CI) −1.45 (−2.79, −0.10)
Heterogeneity: χ²=7.16, df=5 (p=0.21); I²=30%
Test for overall effect: z=2.11 (p=0.03)
−10 −5 0 5 10
Favours metformin Favours control
g
Authors [ref] | Mean difference IV, random, 95% CI
Bugianesi et al, 2005 [45] −19.00 (−30.51, −7.49)
Garinis et al, 2010 [47] −14.00 (−22.32, −5.68)
Haukeland et al, 2009 [43] −1.00 (−4.68, 2.68)
Idilman et al, 2008 [31] −45.00 (−71.89, −18.11)
Lavine et al, 2011 [49] 13.00 (5.74, 20.26)
Nar et al, 2009 [46] 0.00 (−2.71, 2.71)
Omer et al, 2010 [33] 10.00 (0.31, 19.69)
Shields et al, 2009 [44] 2.00 (−25.02, 29.02)
Sofer et al, 2011 [50] −14.00 (−34.25, 6.25)
Tock et al, 2010 [48] −34.00 (−65.11, −2.89)
Uygun et al, 2004 [42] −44.00 (−71.56, −16.44)
Total (95% CI) −7.02 (−13.82, −0.22)
Heterogeneity: τ²=79.45; χ²=64.13, df=10 (p<0.00001); I²=84%
Test for overall effect: z=2.02 (p=0.04)
−50 −25 0 25 50
Favours metformin Favours control
h
Authors [ref] | Mean difference IV, fixed, 95% CI
Bugianesi et al, 2005 [45] −5.00 (−18.82, 8.82)
Garinis et al, 2010 [47] −2.00 (−10.00, 6.00)
Haukeland et al, 2009 [43] −8.00 (−15.43, −0.57)
Idilman et al, 2008 [31] −13.00 (−24.60, −1.40)
Lavine et al, 2011 [49] −6.00 (−15.27, 3.27)
Nar et al, 2009 [46] −11.00 (−31.99, 9.99)
Omer et al, 2010 [33] 6.00 (−1.41, 13.41)
Sofer et al, 2011 [50] −20.00 (−34.82, −5.18)
Tock et al, 2010 [48] −2.00 (−16.20, 12.20)
Uygun et al, 2004 [42] −3.00 (−11.10, 5.10)
Total (95% CI) −4.17 (−7.28, −1.06)
Heterogeneity: χ²=15.90, df=9 (p=0.07); I²=43%
Test for overall effect: z=2.63 (p=0.009)
−20 −10 0 10 20
Favours metformin Favours control
i
Authors [ref] | Mean difference IV, fixed, 95% CI
Haukeland et al, 2009 [43] −0.30 (−0.48, −0.12)
Nar et al, 2009 [46] −0.60 (−1.14, −0.06)
Omer et al, 2010 [33] 0.00 (−0.79, 0.79)
Total (95% CI) −0.31 (−0.48, −0.15)
Heterogeneity: χ²=1.71, df=2 (p=0.43); I²=0%
Test for overall effect: z=3.73 (p=0.0002)
−1 −0.5 0 0.5 1
Favours metformin Favours control
j
Authors [ref] | Mean difference IV, fixed, 95% CI
Garinis et al, 2010 [47] 4.00 (−14.29, 22.29)
Haukeland et al, 2009 [43] −15.00 (−31.62, 1.62)
Lavine et al, 2011 [49] −3.00 (−11.60, 5.60)
Nar et al, 2009 [46] −12.00 (−22.15, −1.85)
Sofer et al, 2011 [50] 2.00 (−6.89, 10.89)
Total (95% CI) −4.10 (−8.95, 0.76)
Heterogeneity: χ²=6.60, df=4 (p=0.16); I²=39%
Test for overall effect: z=1.65 (p=0.10)
−20 −10 0 10 20
Favours metformin Favours control
k
Authors [ref] | Mean difference IV, fixed, 95% CI
Garinis et al, 2010 [47] 7.70 (0.49, 14.91)
Haukeland et al, 2009 [43] 0.80 (−5.13, 6.73)
Lavine et al, 2011 [49] 4.70 (−2.17, 11.57)
Nar et al, 2009 [46] 9.30 (1.18, 17.42)
Sofer et al, 2011 [50] 6.90 (2.95, 10.85)
Total (95% CI) 5.76 (3.17, 8.36)
Heterogeneity: χ²=4.10, df=4 (p=0.39); I²=3%
Test for overall effect: z=4.35 (p<0.0001)
−10 −5 0 5 10
Favours control Favours metformin
l
Authors [ref] | Mean difference IV, fixed, 95% CI
Garinis et al, 2010 [47] 7.00 (−9.63, 23.63)
Haukeland et al, 2009 [43] 2.00 (−8.68, 12.68)
Idilman et al, 2008 [31] −8.00 (−17.52, 1.52)
Lavine et al, 2011 [46] −10.60 (−29.78, 8.58)
Nar et al, 2009 [50] 12.00 (−8.31, 32.31)
Sofer et al, 2011 [42] −15.00 (−28.34, −1.66)
Uygun et al, 2004 5.00 (−10.69, 20.69)
Total (95% CI) −2.85 (−7.97, 2.26)
Heterogeneity: χ²=10.09, df=6 (p=0.12); I²=41%
Test for overall effect: z=1.09 (p=0.27)
−20 −10 0 10 20
Favours metformin Favours control
m
Authors [ref] | Mean difference IV, fixed, 95% CI
Garinis et al, 2010 [47] −1.20 (−2.73, 0.33)
Haukeland et al, 2009 [43] 0.30 (−1.48, 2.08)
Idilman et al, 2008 [31] −3.10 (−5.88, −0.32)
Sofer et al, 2011 [50] 0.00 (−1.98, 1.98)
Total (95% CI) −0.73 (−1.67, 0.22)
Heterogeneity: χ²=4.97, df=3 (p=0.17); I²=40%
Test for overall effect: z=1.51 (p=0.13)
−4 −2 0 2 4
Favours metformin Favours control
n
Authors [ref] | Mean difference IV, fixed, 95% CI
Garinis et al, 2010 [47] 13.10 (3.68, 22.52)
Haukeland et al, 2009 [43] −0.10 (−11.03, 10.83)
Sofer et al, 2011 [50] 11.20 (0.33, 22.07)
Total (95% CI) 8.60 (2.63, 14.56)
Heterogeneity: χ²=3.53, df=2 (p=0.17); I²=43%
Test for overall effect: z=2.82 (p=0.005)
−20 −10 0 10 20
Favours control Favours metformin

Glucose metabolism and cardiovascular risk Metformin significantly reduced body weight, waist circumference, HOMA, FPG, and HbA1c, and increased HDL-C and adiponectin, but had no effect on LDL-C, TG, blood pressure [50] and CRP (Fig. 2). There was no significant publication bias for assessed outcomes (not reported).

Heterogeneity of results for HOMA was abated after excluding trials not effectively implementing lifestyle intervention (as suggested by absence of weight loss in the controls) [43, 46, 49] (WMD −21%, 95% CI −31, −11, p=0.0001, I^2=40%, n comparisons=7), suggesting that the insulin-sensitising effects of metformin are potentiated when lifestyle intervention is effectively implemented.

Metformin was safe and well-tolerated: gastrointestinal intolerance was the most common adverse effect, not requiring treatment discontinuation.

Lipid-lowering drugs

Statins The hepatological safety of statins in NAFLD has been recently recognised and their feared potential for worsening glucose tolerance seems largely outweighed by their cardiovascular benefit [50, 51].

Four RCTs (719 participants, three with a low bias risk) assessed statins in NAFLD [52–55] (ESM Table 3).

Liver disease Statins improved ALT (ESM Fig. 3) and radiological steatosis [53, 54] in hyperlipidaemic NAFLD patients; in the only RCT with post-treatment histology, simvastatin had no effect on liver histology [56]. There was no significant publication bias (ESM Fig. 2)

Glucose metabolism and cardiovascular risk Statins improve LDL-C, HDL-C and TG without affecting body weight (ESM Fig. 3). One RCT found no effect of statins on waist circumference, BP, FPG and CRP [53]. There was no significant publication bias for assessed outcomes (not reported).

In a post hoc analysis of the Greek Atorvastatin and Coronary Heart Disease Evaluation (GREACE) RCT, stain-treated hyperlipidaemic NAFLD patients experienced a significant (−68%) risk reduction of CVD events compared with both presumed NAFLD patients not on statin and with statin-treated patients with normal transaminases, without significant adverse events, including new-onset diabetes [55]. Importantly, this study demonstrates that statins are safe in NAFLD and that statin-related cardiovascular benefit is greater in patients with NAFLD than in those with normal liver tests.

Ezetimibe Growing evidence connects non-esterified cholesterol accumulation in mitochondria to hepatocyte apoptosis, liver injury and NASH development [56–61]. On this basis, ezetimibe, a Niemann-Pick C1 like 1 protein inhibitor, was evaluated in two RCTs in NAFLD.

Liver disease Ezetimibe reduced histological ballooning and fibrosis in one RCT, and MR-assessed liver fat in the other [62, 63] (ESM Table 3).

Glucose metabolism and cardiovascular risk Ezetimibe improved LDL-C and CRP, without affecting weight, waist and HOMA. In one RCT, ezetimibe treatment was associated with significant HbA_{1c} increase compared with placebo [64].

n-3 polyunsaturated fatty acids Five RCTs (303 participants, two RCTs with low risk of bias) evaluated polyunsaturated fatty acids (PUFA) [64–68] (ESM Table 3).

Liver disease PUFA improved ALT (ESM Fig. 4) and steatosis by NMR or ultrasound [65–68]. In the only RCT with post-treatment histology, PUFA ameliorated steatosis without affecting other histological features [68]. There was no significant publication bias (ESM Fig. 2).

Glucose metabolism and cardiovascular risk PUFA ameliorated HOMA, HDL-C and TG, but had no effect on body weight, BP and LDL-C (ESM Fig. 4). One RCT found no effect of PUFA on waist circumference and CRP [68]. There was no significant publication bias for assessed outcomes (not reported).

Overall, PUFA were well-tolerated, with minor gastrointestinal symptoms.

Probucol Probucol, a lipophilic lipid-lowering agent with strong antioxidant activity, was evaluated in NASH in one RCT: ALT improved, but post-treatment histology was unavailable [69] (ESM Table 3). Although generally well-tolerated, probucol was associated with a significant fall in HDL-C.

Fibrates Following consistent anti-steatogenic activity in animal models of NAFLD [70], fibrates were evaluated in five RCTs (315 participants, four RCTs with a low risk of bias) [53, 71–74] (ESM Table 3).

Liver disease Fibrates had no effect on ALT (ESM Fig. 5), radiological steatosis [75] or liver histology [73]. There was no significant publication bias (ESM Fig. 2).

Glucose metabolism and cardiovascular risk Fibrates improved HDL-C and TG, had no effect on body weight, waist, HOMA, FPG and LDL-C (ESM Fig. 5). One RCT showed no effect of fibrates on BP and adiponectin [53]. There was no significant publication bias for assessed outcomes (not reported).

Angiotensin receptor blockers

The modulation of insulin sensitivity, systemic inflammation, hepatic lipogenesis and fibrogenesis by the renin-angiotensin system and the frequent coexistence of hypertension prompted evaluation of angiotensin receptor blockers in NAFLD. In a well-designed RCT on hypertensive NASH, telmisartan (an angiotensin receptor blocker with peroxisome proliferator activated receptor [PPAR]-γ-regulating activity) improved steatosis, necroinflammation, fibrosis, HOMA, TG and total cholesterol more consistently than valsartan, despite similar BP reduction, suggesting that the peculiar PPAR-γ-agonist activity may mediate the more consistent metabolic and histological benefits of telmisartan [75] (ESM Table 4).

In another RCT on hypertensive NAFLD patients, losartan plus simvastatin significantly improved ultrasonographic steatosis, visceral adiposity, HOMA and CRP compared with amlodipine plus simvastatin, despite similar BP reduction [76] (ESM Table 4).

Endocannabinoid receptor antagonists

The cannabinoid type 1 receptor (CB1) receptor antagonist rimonabant experimentally antagonised appetite, caloric intake, hepatic lipogenesis and fibrogenesis and was evaluated in abdominally obese, dyslipidaemic NAFLD patients from the ADAGIO-Lipids trial [77]: rimonabant reversed CT-assessed steatosis in 48% of patients versus 19% on placebo ($p=0.03$) and extensively improved all cardio-metabolic variables (ESM Table 4). Depressive and anxiety disorders were more common with rimonabant (≅2.0% vs 1% with placebo). Concern about psychiatric adverse effects led to withdrawal of rimonabant, but the development of peripherally acting CB1 antagonists is an area of intense research.

Anti-TNF-α agents (pentoxifylline)

The anti-TNF-α agent pentoxifylline has been evaluated in four RCTs in NASH [78–81] (three with low risk of bias, two assessing post-treatment histology) (ESM Table 4).

Liver disease Pooled data analysis showed that pentoxifylline improved histological steatosis and lobular inflammation (ESM Fig. 6). There was no significant publication bias (ESM Fig. 2).

Glucose metabolism and cardiovascular risk Pentoxifylline had no effect on body weight and HOMA (ESM Fig. 6). One RCT found no impact on plasma LDL-C, HDL-C and TG [80]. There was no significant publication bias for assessed outcomes (not reported).

Overall, pentoxifylline was safe and well-tolerated with minor gastrointestinal symptoms.

Ursodeoxycholic acid (UDCA)

Seven RCTs (639 participants, 21% diabetic; three RCTs with post-treatment histology, five RCTs with a low risk of bias) evaluated UDCA in NAFLD (ESM Table 5) [82–88].

Liver disease Overall, UDCA improved ALT but not liver histology (Fig. 7). For ALT and for lobular inflammation, heterogeneity was high and was abated when considering RCT evaluating high-dose (twofold higher than the conventional dose) UDCA or UDCA+vitamin E, showing a modest benefit: for ALT WMD −20 IU/l, 95% CI −37, −3, $p=0.02$, $I^2=40\%$, n comparisons=3; for lobular inflammation OR 2.3; 95% CI 1.1, 5.0; $p=0.03$, $I^2=0\%$, n comparisons=2). There was no significant publication bias (ESM Fig. 2).

Glucose metabolism and cardiovascular risk UDCA improved adiponectin (Fig. 7). For HOMA and FPG heterogeneity was abated after excluding one RCT evaluating the combination of UDCA+vitamin E, the latter potentially worsening HOMA (see below), showing a consistent benefit with UDCA on both HOMA and FPG (for FPG: WMD −6%, 95% CI −9, −2, $p=0.0005$, $I^2=40\%$, n comparisons=3).

One RCT reported significant improvement in HbA_{1c} and HDL-C with high-dose UDCA [85]. There was no significant publication bias for assessed outcomes (not reported).

Minor gastrointestinal effects occurred in >40% of patients on high-dose UDCA.

Semi-synthetic bile acids

Besides their role in nutrient absorption, bile acids are signalling molecules that exert genomic and non-genomic effects by activating TGR5 and farnesoid X receptor (FXR).

TGR5 is a G-protein-coupled receptor (expressed in brown adipose tissue muscle and gut), activation of which by bile acids increases energy expenditure and glucagon-like peptide-1 (GLP-1) secretion and attenuates diet-induced obesity [89, 90].

FXR is a nuclear hormone receptor expressed in the liver, intestine and kidney. In the liver, FXR controls lipogenesis, very-LDL metabolism, gluconeogenesis, glycogen synthesis and insulin sensitivity, and also has also anti-inflammatory and anti-fibrotic properties [90].

On this basis, a novel class of semi-synthetic bile acid agonists of TGR5/FXR is being evaluated for the treatment of obesity-related disorders, including NAFLD.

In the first RCT, Int-747, a semi-synthetic derivative of the human bile acid CDCA, administered for 6 weeks to

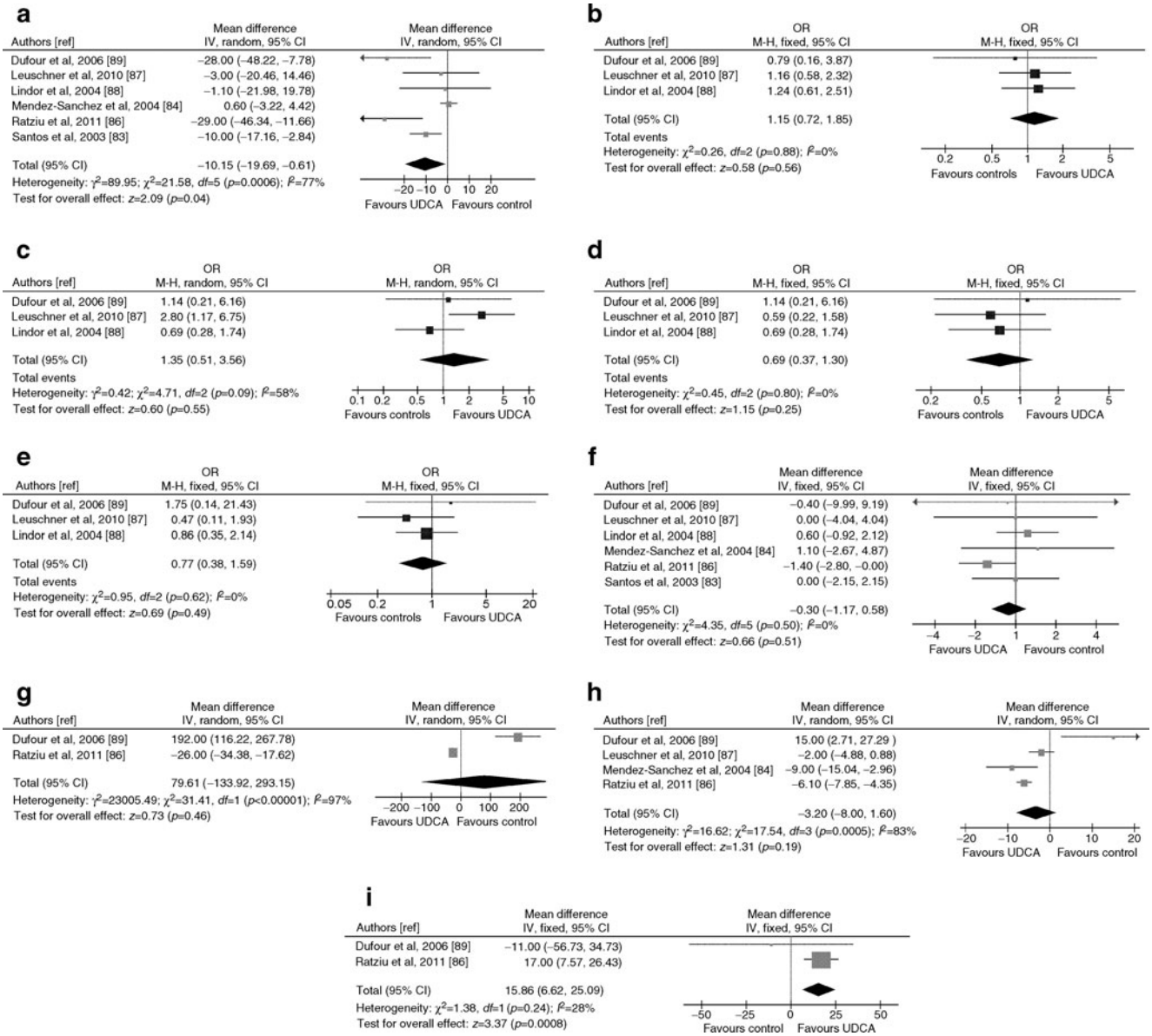


Fig. 7 Forest plots of RCTs comparing the effect of UDCA on liver disease, glucose metabolism and cardiovascular risk. (**a**) Improvement in serum ALT (IU/l). (**b**) Improvement in histological steatosis in NASH. (**c**) Improvement in lobular inflammation in NASH. (**d**) Improvement in hepatocellular ballooning in NASH. (**e**) Improvement in fibrosis in NASH. (**f**) Body weight change (%). (**g**) HOMA index change (%). (**h**) FPG change (%). (**i**) Serum adiponectin change (%). M-H, Mantel–Haenszel

diabetic NAFLD patients, was well-tolerated, ameliorated markers of liver fibrosis, insulin resistance and induced weight loss compared with placebo (ESM Table 4) [91]. The ongoing FXR Ligand NASH Treatment (FLINT) double blind, placebo controlled, multicentre trial is evaluating the effects of obeticholic acid in NASH.

Antioxidants

Fifteen RCTs (1,141 participants, 9% diabetic, seven RCTs with low risk of bias) evaluated antioxidants in NAFLD: overall, heterogeneity in study population, duration, type and dose of drug, lifestyle intervention implementation, was considerable [6, 32, 49, 92–99] (ESM Table 5).

Liver disease Pooled results of the seven RCTs (685 patients, 4% diabetic) with post-treatment histology showed no histological improvement and high heterogeneity (Fig. 8). Heterogeneity was reduced when considering only the five RCTs with vitamin E, showing modest improvement in steatosis (OR 1.83; 95% CI 1.03, 3.25; I^2=35%, p=0.04) and lobular inflammation (OR 1.57; 95% CI 1.03, 2.39; I^2=0%, p=0.04). One RCT reported also an improvement in NAS score with Viusid [98].

Antioxidants as a group or vitamin E did not slow fibrosis progression (Fig. 8).

Predictors of histological response to antioxidants are unclear: weight loss, circulating oxidative stress markers or vitamin E deficiency do not predict histological response [49, 95, 96, 100]. There was no significant publication bias (ESM Fig. 1)

Glucose metabolism and cardiovascular risk Antioxidants had no effect on body weight, waist circumference, LDL-C and HDL-C. For HOMA, FPG and TG heterogeneity was high (Fig. 8): when considering only the RCTs with vitamin E, active treatment had no significant effect on FPG (WMD −0.04, 95% CI −0.66, 0.57, p=0.89, I^2=0%, n comparisons=5), but was associated with a modestly higher risk of increasing HOMA (WMD 10.5, 95% CI 0.3, 20.6, p=0.04, I^2=45%, n comparisons=4) and plasma TG (WMD 6.00, 95% CI 1.26, 10.75, p=0.01, I^2=0%, n comparisons=4) compared with controls. One RCT showed an improvement in plasma adiponectin with the combination of UDCA+vitamin E compared with placebo [88]. There was no significant publication bias for assessed outcomes (not reported).

Other drugs: L-carnitine, probiotics, incretin analogues, caspase inhibitors

L-carnitine is a precursor of carnitine-palmitoyltransferase, the rate-limiting enzyme in mitochondrial fatty acid transport and oxidation. When added to lifestyle intervention for 6 months, it improved steatosis, inflammation, fibrosis, HOMA, FPG, plasma lipids and C-reactive protein (ESM Table 5) [101].

Gut bacteria may promote liver injury and systemic inflammation through endotoxin-mediated toll-like receptor-4 axis activation and other mechanisms, predisposing to NASH, diabetes and atherosclerosis [102]. Three RCTs assessed probiotics in NAFLD: the first, evaluating VSL3, was prematurely terminated for an increase in hepatic steatosis [103]; the others, assessing a mixture of *Lactobacillus* spp. plus either *Bifidobacterium bifidum* or *Streptococcus thermophilus*, found a significant improvement in MRS-assessed hepatic fat and aminotransferases, respectively [104, 105].

The effect of probiotics in NAFLD is being evaluated in clinical trials (trial registration clinicaltrials.gov NCT00099723, NCT00808990, NCT00870012).

Incretin GLP-1 analogues improved insulin secretion by stimulating pancreatic beta cell growth and insulin release, and also improved hepatic steatosis and insulin resistance in mouse models [106]. Exenatide significantly improved transaminases in three RCTs enrolling diabetic patients [107], and its effects on liver histology in NASH are being tested in clinicaltrials.gov NCT00529204 and NCT00650546. In the Liraglutide Effect and Action in Diabetes (LEAD)-2 RCT, 2 years of liraglutide significantly reduced liver enzymes, CT-assessed hepatic steatosis, body fat and blood pressure and improved glycaemic control in diabetic patients with NAFLD (ESM Table 5) [108].

In NASH, hepatocyte apoptosis correlates with disease severity, and reducing hepatocyte apoptosis may has a potential for altering the course of the liver disease. In a phase 2 RCT, 124 patients with biopsy-proven NASH were randomised to once-daily placebo or 1, 5, 10 or 40 mg of the selective caspase inhibitor GS-9450 for 4 weeks: at EOT, NASH patients treated with 5–40 mg/day of GS-9450 significantly improved ALT levels, but not other metabolic variables, without significant side effects [109].

Discussion

Implications for practice

Weight loss is safe and may benefit both liver and cardio-metabolic disease in NAFLD: although a ≥5% weight loss improves steatosis and cardio-metabolic variables, a ≥7% weight loss improves also histological disease activity in NASH; however, the latter goal was achieved by <50% individuals even in RCTs adopting intensive multidisciplinary lifestyle interventions, making patient compliance a concern [8, 10].

Regular moderate-intensity aerobic exercise should be implemented in lifestyle intervention, as it enhances whole body lipid oxidation, and improves steatosis and cardio-metabolic risk profile regardless of weight loss: it may also protect NAFLD patients against the development of diabetes [110].

For patients with NASH not responding to lifestyle intervention, pharmacological treatment should be considered. Currently, no specific pharmacological treatment can be recommended outside clinical trials, for long-term safety and efficacy concerns. Among available agents, TZDs, statins, PUFA and antioxidants have been most extensively evaluated. Statins and PUFA ameliorate steatosis and liver enzymes, but their impact on liver histology is unknown,

TZDs improve steatosis and necroinflammation, slow fibrosis progression, and ameliorate glucose and lipid metabolism and subclinical inflammation, with more consistent cardiovascular benefits with pioglitazone. These findings should not be underestimated, given the key role of fibrosis in the progression of NAFLD to end-stage liver disease, and pioglitazone warrants evaluation in a larger RCT of longer duration. Open issues on TZDs are their long-term safety and efficacy, how to prevent their side effects and the development of predictors of histological response to these drugs.

Antioxidants yielded mixed results on liver histology, improving histological disease activity when administered for 2 years or when implemented with vigorous weight-loss regimens [97].

Differently from TZDs, vitamin E worsened insulin resistance and plasma TG. Several studies found that vitamins E may preclude the insulin-sensitising effects of exercise by hampering physiological training-induced cellular adaptations in muscle in healthy individuals: vitamin E supplementation prevented exercise-induced production of PPAR-γ, PPAR-γ coactivators PGC1α and PGC1β, and antioxidant enzymes superoxide dismutase and glutathione peroxidase [111]. Although these data have not been recently confirmed [112, 113], the impact of antioxidants on muscle insulin sensitivity in insulin-resistant individuals is unclear. An increased all-cause mortality has been associated with long-term administration of doses of vitamin E typically used in these trials [114]. Finally, antioxidant effectiveness in diabetic NAFLD patients, characterised by prominent systemic oxidative stress and severe liver histology, is unknown, as only 9% of enrolled patients were diabetic.

Implications for future research

With the exception of the GREACE trial [55] , no RCT had adequate size and duration to evaluate clinical outcomes. Therefore, future RCTs need to assess if the observed benefits on intermediate endpoints will translate into a reduction of liver-related and cardio-metabolic morbidity and mortality.

The optimal dietary nutrient composition for NAFLD is an uncovered field: the role of excessive fructose, cholesterol and *trans* fat for NAFLD pathogenesis, as suggested by epidemiological and experimental studies, deserves evaluation in therapeutic RCTs. Fructose and high-fructose corn syrup, a common soft drink sweetener, in particular, have been independently connected to the risk and severity of NAFLD in population-based studies and in a randomised crossover trial [115–119].

The role of alcohol consumption in NAFLD needs also further evaluation: retrospective data suggest a protective role for light-to-moderate (<10–20 g/day) alcohol intake against insulin resistance, metabolic syndrome and NAFLD [120, 121]. By contrast, modest alcohol intake and obesity seem to have additive effect on liver disease progression, and in a large prospective study any degree of alcohol consumption increased by 3.6-fold the risk of hepatocellular carcinoma in NASH-related cirrhosis [122, 123].

Cigarette smoking, an established risk factor for CVD and metabolic syndrome, has been epidemiologically linked to the onset and severity of NASH [124–126]. In the GREACE trial [54], current smokers had an OR of having baseline abnormal liver enzymes of 3.03 (95% CI 1.99, 4.64) compared with non-smokers. These data prompt evaluation of the effects of smoking cessation on NAFLD in future RCTs.

With the possible exceptions of telmisartan and pentoxifylline (limited evidence from two small RCTs), available agents do not improve hepatic fibrosis, the features most consistently associated with adverse liver-related outcomes. This may have several explanations: the slower progression rate (0.1–0.2 stages per year) of fibrosis [127] may require larger and longer RCTs to show fibrosis regression, and the encouraging results of TZDs on fibrosis progression are consistent with this view; alternatively, hepatic fibrogenesis may involve different molecular mechanisms from those involved in dysmetabolism, steatosis and inflammation. Within this context, antifibrotic agents targeting directly hepatic stellate cell activation and collagen deposition/remodelling, including toll-like receptor-4, tissue inhibitors of metalloproteinases (TIMPs) and FXR, are under development and phase III RCTs are eagerly awaited [128].

Our analysis provides also some hints for methodological improvement of future RCTs. Concerning cardio-metabolic risk of NAFLD, it is currently unclear whether NAFLD determines or is just a marker of associated cardio-metabolic abnormalities, and a comprehensive cardio-metabolic profiling of these patients may help predicting the impact of proposed treatments on cardio-metabolic outcomes [129]. As an example, HbA_{1c} (reported in only 22% of RCTs; Table 1) is emerging as a robust marker not only of recent glycaemic control in diabetes, but also of the risk of developing diabetes and CVD in diabetic and non-diabetic individuals [130, 131].

The risk of developing or deteriorating type 2 diabetes is related to insulin resistance and pancreatic beta cell dysfunction [132]. In NAFLD, insulin resistance is universal, but impaired pancreatic beta cell function was also found in non-diabetic patients with NASH [133]. The different tissue insulin sensitivity also needs attention. Most RCTs adopted fasting insulin sensitivity indices (HOMA and QUICKI) (Table 1), which are easy to measure, predict incident CVD and diabetes in the general population and overall mortality in NAFLD [134, 135], but may have some limitations in such a complex disease as NAFLD. Insulin sensitivity is tissue-specific and skeletal muscle (i.e. the ability of insulin to enhance glucose disposal in muscle), hepatic (i.e.

Fig. 8 Forest plots of RCTs comparing the effect of antioxidants on liver disease, glucose metabolism and cardiovascular risk. (**a**) Improvement in histological steatosis in NASH. (**b**) Improvement in lobular inflammation in NASH. (**c**) Improvement in hepatocellular ballooning in NASH. (**d**) Improvement in fibrosis in NASH. (**e**) Improvement or stability in fibrosis in NASH. (**f**) Body weight change (%). (**g**) Waist circumference change (%). (**h**) HOMA index change (%). (**i**) FPG change (%). (**j**) Plasma LDL-cholesterol change (%). (**k**) Plasma HDL-cholesterol change (%). (**l**) Plasma TG change (%).M-H, Mantel–Haenszel

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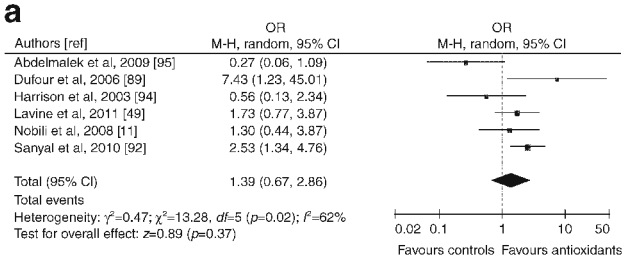

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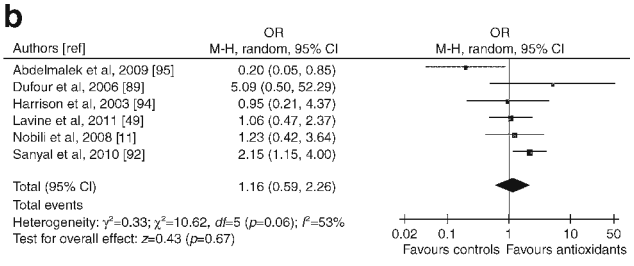

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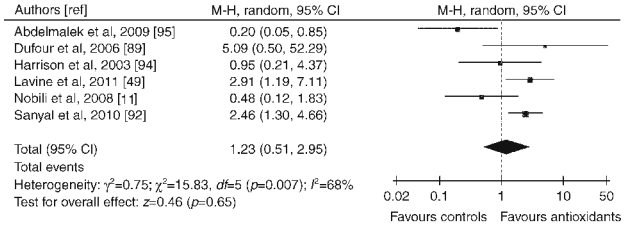

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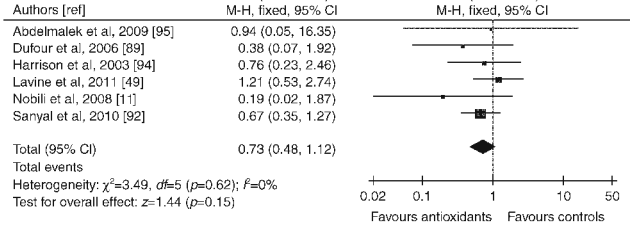

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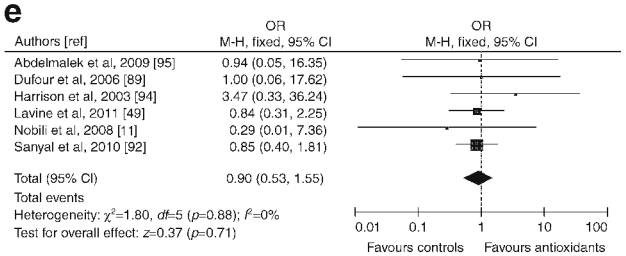

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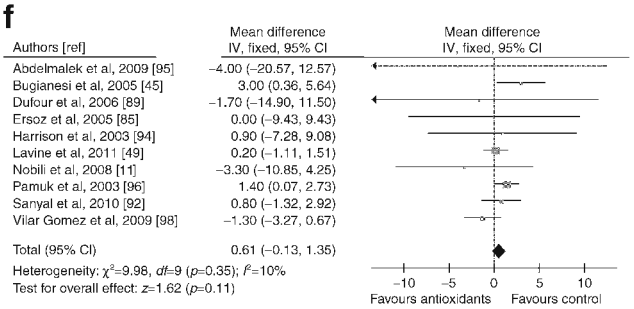

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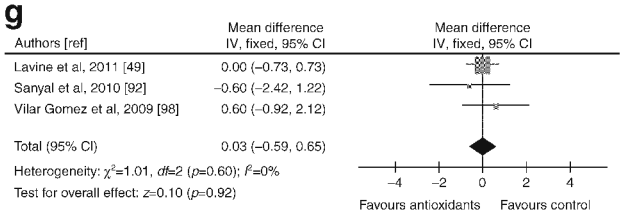

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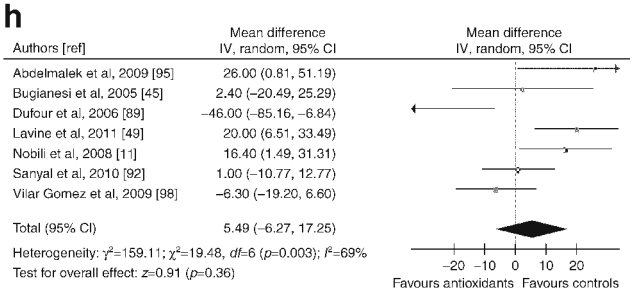

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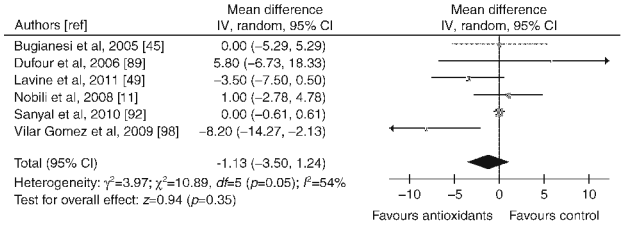

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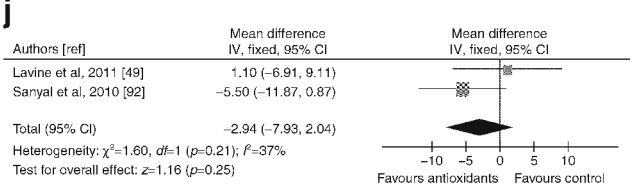

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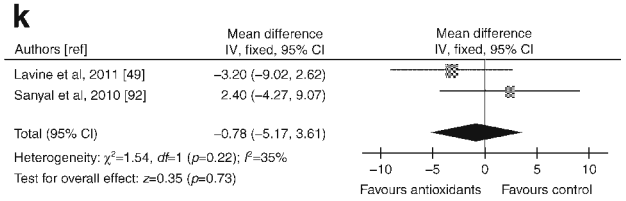

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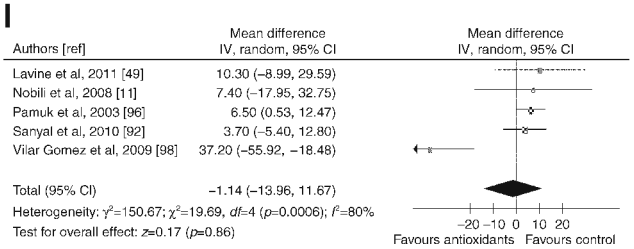

the ability of insulin to suppress hepatic glucose output in fasting conditions) and adipose tissue (i.e. the ability of insulin to suppress adipose tissue lipolysis) insulin sensitivity do not always parallel each other and may differently relate to liver and cardio-metabolic disease: whereas liver injury seems tightly related to adipose tissue insulin sensitivity in NASH [26], hepatic or muscle insulin sensitivity are more tightly related to glucose tolerance and the risk of future diabetes [136]. This may explain why metformin does not affect liver histology despite constant HOMA reduction and, similarly, the lack of improvement in liver injury despite continued HOMA improvement observed in the FLIRT trials. Therefore, different tissue insulin sensitivity should be systematically assessed, together with pancreatic beta cell function, with a simple standard OGTT, without applying the more troublesome glucose clamp technique [137].

Plasma inflammatory markers are also emerging as important tools in risk assessment and targeting of therapy in patients with metabolic syndrome and could be extended to RCTs on NAFLD [138].

In conclusion, weight loss and pioglitazone seem to most extensively benefit intermediate endpoints in NAFLD, improving not only liver disease but also cardio-metabolic variables [139], while vitamin E improves histological disease activity but may worsen the cardio-metabolic profile. The latter issue, as well as the risk/benefit profile of other antioxidants in NAFLD [140], needs further evaluation in future RCTs adequately powered for clinical outcomes.

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Contribution statement GM conceived and designed the study, analysed data, discussed results, wrote the manuscript and approved the final version of the manuscript. MC, FR and RG analysed data, revised the manuscript critically for important intellectual content and approved the final version of the manuscript.

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