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Meta-Analysis of Randomized Controlled Trials of Pharmacologic Agents in Non-alcoholic Steatohepatitis

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ABSTRACT

Background. Currently, there is no standardized treatment regimen for non-alcoholic steatohepatitis. **Aim.** We performed a meta-analysis of high quality randomized controlled trials that evaluated treatment response to metformin, thiazolidinediones (TZDs), and vitamin E in adult patients with non-alcoholic steatohepatitis. Outcome measures were improvement in liver histology, biochemical, and anthropometric measures. **Material and methods.** Nine trials met inclusion criteria (3 with TZD, 3 with Metformin, 2 with Vitamin E and 1 with both TZD and Vitamin E.). **Results.** With metformin, weighted liver histologic scores for steatosis, ballooning, and fibrosis did not demonstrate significant improvement and lobular inflammation worsened significantly (weighted mean increase 0.21, 95% CI 0.11 to 0.31, P < 0.0001). The liver histology score including steatosis (OR 3.51, 95% CI 2.14 to 5.78) and lobular inflammation (OR 2.65, 95% CI 1.69 to 4.15) improved with TZDs. Hepatic fibrosis (OR 1.58, 95% CI 0.98 to 2.54) and ballooning scores (OR 1.84, 95% CI 0.94 to 3.58) did not demonstrate significant improvement. With Vitamin E, weighted liver histologic scores for steatosis (weighted mean decrease -0.60, 95% CI -0.85 to -0.35, P < 0.0001), lobular inflammation (weighted mean decrease -0.40, 95% CI -0.61 to -0.20, P = 0.0001) and ballooning (weighted mean decrease -0.30, 95% CI -0.54 to -0.07, P = 0.01) demonstrated significant improvement compared to placebo. Fibrosis did not significantly change. **Conclusion.** In patients with NASH, TZDs and Vitamin E improve liver histologic scores but metformin does not. Insulin resistance also improves with both TZDs and metformin. Fibrosis does not improve with any of the agents.

Key words. NASH (Non Alcoholic Steatohepatitis). NAFLD (Non Alcoholic Fatty Liver Disease). Rosiglitazone. Pioglitazone. Metformin. Vitamin E. Histology. Meta-analysis. Systematic review.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) encompasses a spectrum of disease ranging from steatosis to steatohepatitis and cirrhosis. It is considered the most common cause of elevated liver enzymes with an estimated twenty percent of the population affected worldwide.[1-4] Non-alcoholic steatohepatitis (NASH) refers to a subset of patients with NAFLD who demonstrate histological characteristics of hepatic steatosis, lobular inflammation and hepatocellular ballooning with progression to cirrhosis estimated in up to fifteen percent of patients within a ten year period.[1,2,5,6] In comparison to matched controls patients with either NAFLD or NASH have an increased mortality rate likely secondary to the prevalence of cardiovascular disease present in this population.[1,2] Also, NASH cirrhosis has been associated with the development of hepatocellular carcinoma.[7] Given the increasing incidence of NAFLD and the long-term consequences of this disease it is important to identify the risk factors and therapeutic measures, which can help curtail the progression of this aggressive illness.

The pathogenesis of NAFLD is not well understood, however, insulin resistance and metabolic syndrome have been associated with NAFLD.[8] Also, lipid peroxidation and its' byproducts have been found to be elevated in NASH patients and correlate directly to increasing necroinflammatory activity and fibrosis.[9] Consequently, drugs that counteract these specific mechanisms have been used to facilitate the reduction of inflammation and fibro-

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sis found in the liver of patients afflicted with NAFLD and NASH. Currently, there is no defined regimen for the treatment of NAFLD though many treatments have been purported.

Studies involving thiazolidinediones (TZDs), metformin, and anti-oxidants have been shown to improve biochemical parameters, glucose, and lipid metabolism.[8,10-13] On the other hand, histological improvement with the same treatment measures has been difficult to interpret. A study by Bugianesi, *et al.* demonstrated metformin decreased the percentage of hepatic steatosis, necroinflammation, and fibrosis in non-diabetic patients, whereas a pilot study of metformin did not demonstrate a significant difference in any histopathological parameters when compared to placebo.[14] Sanyal, *et al.* demonstrated improvement in lobular inflammation and hepatic steatosis but no significant improvement in fibrosis with pioglitazone or vitamin E.[13] However, a placebo controlled trial of pioglitazone in non-diabetic patients' demonstrated improvement in fibrosis over a twelve-month period.[15] A recent meta-analysis by Singh, *et al.* aimed to compare the effectiveness of pharmacological interventions for NASH.[16] However, their analysis excluded the use of metformin and included a Bayesian network analysis which compared agents that have never been tested head to head (Obeticholic acid from a single phase two trial and Pioglitazone). In addition, the pediatric population was also included by Singh, *et al.* which increases the heterogeneity of the studies used and makes analysis more difficult to compare.

Given the limited data and uncertainty regarding the superiority of any pharmacological agent in patients with NASH, we undertook meta-analysis of randomized placebo controlled trials that examined metformin, TZDs such as pioglitazone and rosiglitazone, and vitamin E on adult patients with NASH.

MATERIAL AND METHODS

Study selection

We selected studies using the following databases to Dec 31, 2014 (Figure 1): PubMed, Medline, clinicaltrials.gov, Cochrane central register of controlled trials. Key words used were the following: NASH (Non Alcoholic Steatohepatitis), NAFLD (Non Alcoholic Fatty Liver Disease), Rosiglitazone, Pioglitazone, Metformin, Vitamin E, and histology. We included human studies without language restrictions. Bibliographies or original articles were also searched to identify other relevant articles.

Inclusion criteria for meta-analysis were adult randomized placebo controlled trials in patients with NASH and a minimum duration of therapy of at least six months

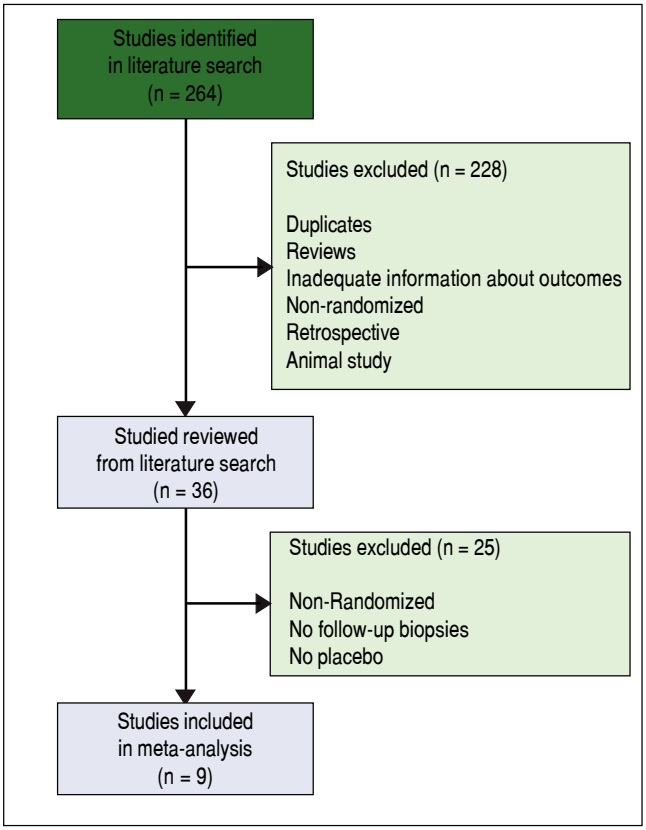


Figure 1. *Flow Chart of Study Information.*

with reportable histology outcomes pre and post-treatment. Patients with diabetes and non-diabetics were included. Although changes in biochemical variables (AST ALT, hyperglycemia) and anthropometric parameters were also analyzed when present, we did not include trials that did not include liver histology outcomes. We did not include trials in children and trials without controls were also excluded. Two investigators independently carried out literature search and reviewed studies for inclusion and exclusion criteria. Data were abstracted independently by two investigators.

Initially, there were thirty-six studies examining the effects of the above pharmacological interventions in patients with NALFD or NASH. As such, only nine total trials fit all inclusion criteria including three trials of TZDs, three of metformin, two of vitamin E and one with both TZD and Vitamin E were included in the analysis.

Outcome measures

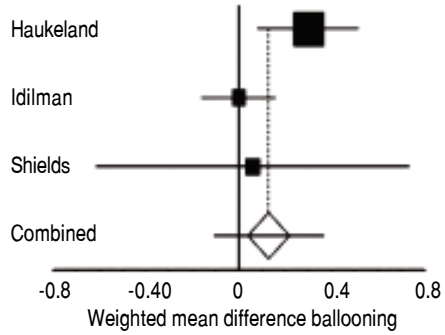
Outcome measures were changes in liver histology including steatosis, ballooning, lobular inflammation, fibrosis and NASH activity index using standardized

Table 1. Review of various randomized controlled trials for non-alcoholic fatty liver disease.

Study	Total N*	Duration Study (months)	Dose of agent used	Study design	Diabetics included	Age of treatment group (s.d. / range)	Age of placebo group (s.d. / range)	Sex of treatment group (M:F)	Sex of placebo group (M: F)	Duration of follow up (months)
Thiazolidinediones										
Aithal, *Gastroenterology* 2008	74	12	Pioglitazone 30 mg/day	Randomized, Placebo Controlled	No	52 (28-71)	55 (27-73)	26:11	19:18	12 months (end of trial)
Belfort, *NEJM* 2006	47	6	Pioglitazone 30 mg/day, increased after 2 months to 45 mg/day	Randomized, Placebo Controlled	Yes (Diabetes or abnormal Glucose Tolerance Test)	51 ± 7	51 ± 10	14:12	7:14	6 months (end of trial)
Sanyal, *NEJM* 2010	163	24	Pioglitazone 30 mg/day	Randomized, Placebo Controlled	No	47 (12.6)	45.4 (11.2)	33:47	35:48	24 months
Ratziu, *Gastroenterology* 2008	63	12	Rosiglitazone 4 mg/day for one month, then 8 mg /day	Randomized, Placebo Controlled	Yes (32%)	53.1 (11.5)	54.1 (10.4)	19:13	18:13	16 months (4 months after end of therapy)
Metformin										
Haukeland, *Scan J Gastro* 2009	44	6	Metformin started at 500 mg/day, maximum dose of 3,000 mg	Randomized Placebo Controlled Trial	Yes (27%)	44.3 (9.0)	49.9 (12.8)	16:4	16:8	6 months (end of treatment)
Idilman, *Alimentary Pharmacol Ther* 2008	18	12	Metformin 850 mg twice daily	Randomized Controlled Trial	Unclear	47.9 (8.3)	45.8 (10.4)	21:27	9:16	12
Shields, *Therapeutic Adv. in Gastro* 2009	19	12	Metformin 500 mg daily titrated to 1,000 mg daily	Randomized Placebo Controlled Trial	No	50.2 (9.1)	44.4 (12)	8:1	5:5	12
Vitamin E										
Sanyal, *NEJM* 2010	167	24	Vitamin E 800 IU/day	Randomized, Placebo Controlled	No	46.6 (12.1)	45.4 (11.2)	32:52	35:48	24 months
Dufour, *Clin Gastro Hepatol* 2006	30	24	Vitamin E 800 IU/day + ursodiol 12-15 mg/kg/day	Randomized Placebo Controlled Trial	Yes	46 (14)	44 (14)	10:5	8:7	24
Harrison, *American J Gastro* 2003	45	6	Vitamin E 1,000 IU/day + Vitamin C 1,000 mg/day	Randomized Placebo Controlled Trial	Yes	52.5	50.2	9:14	11:11	6

N: number of patients.*

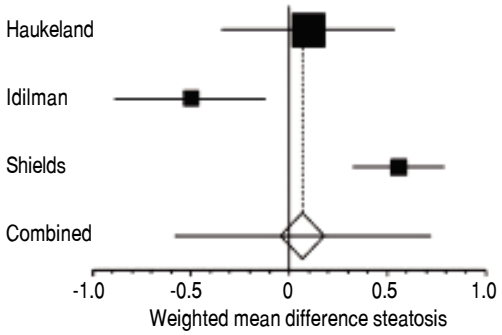
A. Ballooning



Metformin (N)	Placebo (N)	Weighted mean difference (95% CI)
39	42	0.13 (-0.11 to 0.37)

Heterogeneity: χ^2 -4.74, d.f. = 2, P = 0.09, I^2 = 57.8%
Test for overall effect: Z = 1.06, P = 0.29

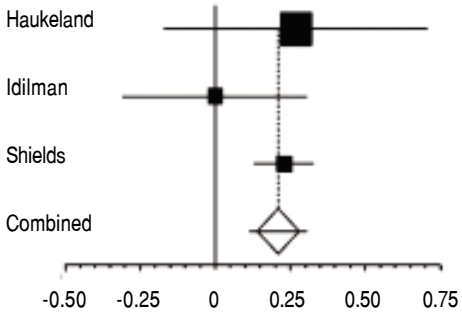
B. Steatosis



Metformin (N)	Placebo (N)	Weighted mean difference (95% CI)
39	42	0.07 (-0.59 to 0.72)

Heterogeneity: χ^2 = 20.5, d.f. = 2, P = < 0.0001, I^2 = 90.3%
Test for overall effect Z = 0.20, P = 0.84

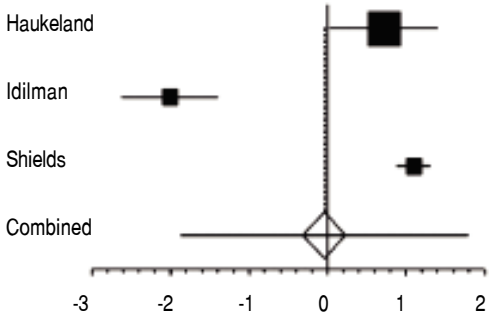
C. Lobular Inflammation



Metformin (N)	Placebo (N)	Weighted mean difference (95% CI)
39	42	0.21 (0.11 to 0.31)

Heterogeneity: χ^2 = 2.03, d.f.= 2, P = 0.36, I^2 = 1.4%
Test for overall effect Z = 4.28, P = < 0.0001

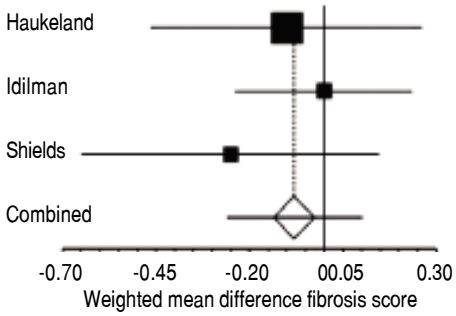
D. NAFLD activity score



Metformin (N)	Placebo (N)	Weighted mean difference (95% CI)
39	42	-0.05 (-1.88 to 1.78)

Heterogeneity: χ^2 = 78, d.f. = 2, P = < 0.0001, I^2 = 97.4%
Test for overall effect Z = -0.05, P = 0.96

E. Fibrosis



Metformin (N)	Placebo (N)	Weighted mean difference (95% CI)
39	42	-0.08 (-0.26 to 0.10)

Heterogeneity: χ^2 = 1.11, d.f.= 2, P = <0.78, I^2 = 0%
Test for overall effect Z = -0.86, P = 0.39

Figure 2. *Metformin and Histological changes in NAFLD.*

histological criteria for NASH reported in the included studies. Also, evaluation of biochemical and anthropometric measures were examined including alanine aminotransferase (ALT), homeostatic model assessment of insulin resistance (HOMA-IR), fasting blood sugar, Hemoglobin A1c (HbA1c), body mass index (BMI), body weight, total cholesterol, high-density lipoprotein (HDL) and triglycerides.

TZD- histology was expressed in dichotomous format (improvement versus not) with Odds ratio and Confidence intervals calculated for each histologic parameter versus placebo.

Metformin and Vitamin E - Histologic parameters were expressed as continuous variables using weighted mean differences and confidence intervals calculated for each histologic parameter versus placebo/controls. Biopsy specimens in the included studies were assessed using the following scoring systems: Brunt (3), Promrat (2), Kleiner (4).

Quality assessment

We assessed the methodological quality of the articles using The Cochrane Collaboration's Tool for Assessing Risk of Bias. We also used the Jadad 3 point scale for assessing the quality of each randomized trial.

Meta-analysis

A meta-analysis of data from randomized controlled trials was performed using Stats Direct Software. For liver histologic parameters, treatment effects for dichotomous data (improvement in liver histology Yes *vs.* No) were expressed by Odds ratio and 95% confidence intervals. For liver histologic parameters, treatment effects for continuous data (change in liver histology parameter scores) were expressed by weighted mean differences (and confidence interval) in each histologic parameter.

Random effects models with the Mantel-Haenszel method were used for combining data from trials. Heterogeneity of trials was assessed using the I^2 measure of inconsistency and the Cochran Q statistic. Publication bias was assessed using funnel plot analysis and the Egger test.

Continuous variables

For continuous biochemical and anthropometric data weighted averages were estimated utilizing study means, sample size, and standard deviations. The Fisher exact method was used to combine the individual study P values and calculate an overall P value for comparison of each of these parameters.

RESULTS

Metformin

Three trials analyzing metformin therapy in patients with NASH were included in our meta-analysis (Table 1) with only Haukeland, *et al.*[17] including a subset of diabetic patients. 81 patients were analyzed in the metformin group

Table 2. Effect of metformin on biochemical and anthropometric variables in NAFLD.

Variable	Metformin (n = 129) Control (n = 103)	Weighted means pre-treatment	Weighted means post-treatment	P values within group	P value metformin *vs.* controls
Blood Sugar	Metformin Control	98.43 99.72	87.93 99.07	< 0.0001 0.002	0.003
HOMA-IR	Metformin Control	3.52 3.07	2.80 3.47	0.0004 0.0016	< 0.0001
Triglycerides	Metformin Control	184.65 178.25	184.45 173.83	0.0004 0.0055	0.08
Total Cholesterol	Metformin Control	208.55 206.95	189.40 187.55	0.0001 < 0.0001	0.0008
ALT	Metformin Control	95.48 92.65	52.09 69.25	< 0.0001 < 0.0001	< 0.0001
Body Weight (kg)	Metformin Control	85.31 84.41	82.78 88.62	< 0.0001 0.054	< 0.001
BMI	Metformin Control	30.51 30.02	28.74 28.92	< 0.0001 < 0.0001	< 0.0001

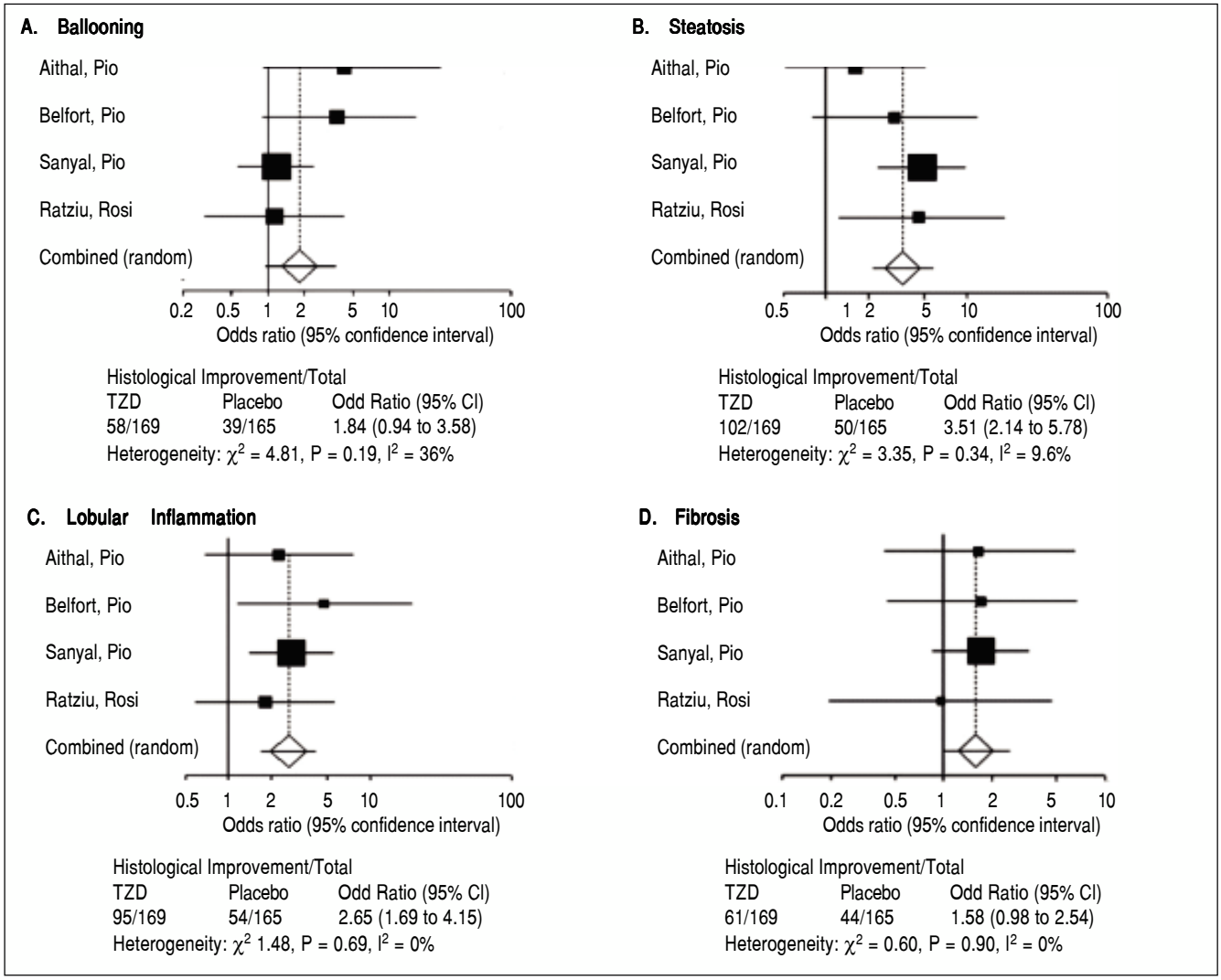


Figure 3. *Thiazolidinediones and histological changes in NAFLD.*

with over half (54%) attributable to the Haukeland, *et al.* study.[17] Therapy lasted between six to twelve months. Of note, therapy regimens varied with Metformin dosed between 1,000 mg to 3,000 mg daily. Histological parameters including ballooning, fibrosis, steatosis and NAFLD activity score (NAS) did not significantly change with metformin therapy (Figure 2). Lobular inflammation significantly worsened after therapy (Figure 2) (weighted mean increase 0.21, 95% CI 0.11 to 0.31, P < 0.0001). Biochemical parameters including fasting blood sugar, HOMA-IR, total cholesterol, ALT, body weight and BMI all significantly improved with metformin therapy as compared to the control group (Table 2). Triglyceride levels did not improve significantly with metformin. Of note, blood sugar, triglycerides, total cholesterol, ALT, and BMI all significantly improved within the control group during the trial; HOMA-IR significantly worsened within the control group.

Thiazolidinediones

Four trials analyzing TZD (3 pioglitazone and 1 rosiglitazone) therapy in patients with NASH were included in our meta-analysis (Table 1) with Belfort, *et al.*[18] and Ratziu, *et al.*[19] including a subset of diabetic patients. 347 patients were analyzed in the TZD group with studies ranging from 47 to 163 patients. Therapy lasted between six to twenty four months. Of note, therapy regimens varied including Pioglitazone 30 mg daily with up-titration to 45 mg daily as well as Rosiglitazone up to 8 mg daily. Histological parameters including ballooning and fibrosis did not significantly change with

Table 3. Effect of Thiazolidinediones on Biochemical and Anthropometric Variables in NAFLD.

Variable	TZD (n = 175) Controls (n = 172)	Weighted Means Pre-Treatment	Weighted Means Post- Treatment	P Values Within Group	P value TZD *vs.* Controls
Hemoglobin A1c	TZD	5.77	5.55	< 0.0001	< 0.0001
	Control	5.86	5.87	0.007	
Blood Sugar	TZD	95.79	92.29	0.0007	< 0.0001
	Control	99.60	102.5	0.0003	
HOMA-IR	TZD	4.60	3.62		< 0.0001
	Control	5.01	5.18		
Triglycerides	TZD	142.2	129.4	0.05	0.0006
	Control	166.7	162.9	< 0.0001	
High-density lipoprotein	TZD	44.7	46.9	0.0007	0.005
	Control	42.9	43.7	0.08	
Total Cholesterol	TZD	192.3	186.1	0.04	0.0015
	Control	199.53	193.1	0.03	
ALT	TZD	71.96	35.12	< 0.0001	< 0.0001
	Control	75.87	50.55	0.0001	
Body Weight (kg)	TZD	90.90	94.25	< 0.0001	< 0.0001
	Control	93.15	91.91	0.16	
BMI	TZD	31.34	32.70	0.0001	0.0005
	Control	31.78	32.38	0.28	

HOMA-IR: Homeostatic model assessment of insulin resistance. ALT: Alanine aminotransferase. BMI: Body mass index.

TZD therapy (Figure 3). Steatosis and lobular inflammation significantly improved after therapy (Figure 3). Biochemical parameters including HbA1c, fasting blood sugar, HOMA-IR, triglycerides, total cholesterol, high-density lipoprotein, and ALT all significantly improved with TZD therapy as compared to controls (Table 3). High-density lipoprotein, body weight, and BMI significantly worsened with TZD therapy as compared to controls. Of note, triglycerides, total cholesterol, and ALT all significantly improved within the control group. HbA1c and fasting blood sugar significantly worsened with the control group. High-density lipoprotein, body weight, and BMI did not significantly change in the control group.

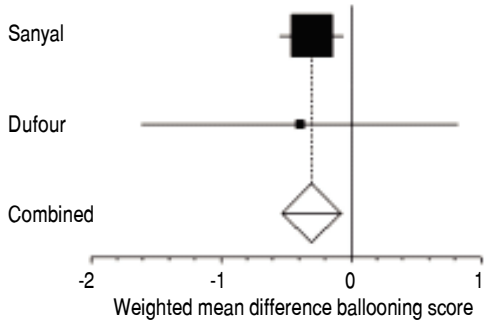
Vitamin E

Three trials analyzing vitamin E therapy in patients with NASH were included in our meta-analysis (Table 1) with Harrison, *et al.*[20] and Dufour, *et al.*[21] including a subset of diabetic patients. 242 patients were analyzed in the Vitamin E group with studies ranging from 30 to 167 patients, with the predominant number of patients coming from the Sanyal, *et al.* study.[13] Therapy lasted between six to twenty four months. Of note, therapy regimens varied with the use of Vitamin E 800 to 1000 IU/day. Histological parameters including fibrosis and NAS did not significantly change with vitamin E therapy (Figure 4). Ballooning, steatosis, and lobular inflammation significantly improved with vitamin E (Figure 4). Analysis of biochemical parameters within the vitamin E group was limited secondary to reported data, as such, only ALT and BMI changes were evaluated (Table 4). ALT significantly improved with vitamin E therapy as compared to the control group. BMI did not significantly change with vitamin E therapy as compared to the control group. Of note, ALT and BMI did significantly improve within the control group as well.

DISCUSSION

Our meta-analysis of high quality randomized controlled trials demonstrates that metformin, TZDs, and Vitamin E therapy do not significantly improve fibrosis. However, both TZDs and Vitamin E significantly improve steatosis and lobular inflammation with Vitamin E also significantly improving hepatocyte ballooning. All of the agents studied significantly improved ALT. Metform-

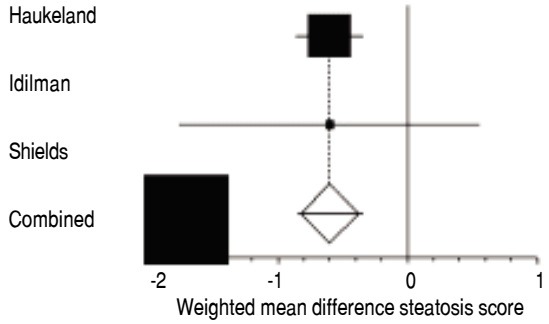
A. Ballooning



Vitamin E (N)	Placebo (N)	Weighted mean difference (95% CI)
94	94	-0.30 (-0.54 to -0.07)

Heterogeneity: χ^2 = 0.02, d.f. = 1, P = 0.89
Test for overall effect Z = -2.50, P = 0.01

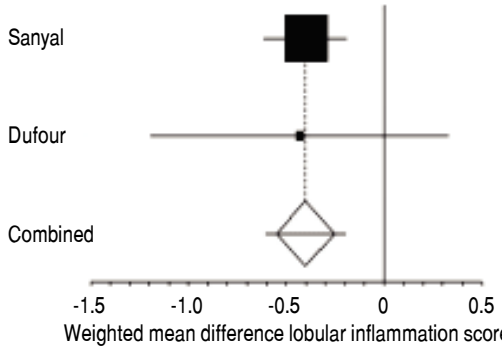
B. Steatosis



Vitamin E (N)	Placebo (N)	Weighted mean difference (95% CI)
94	94	-0.60 (-0.85 to -0.35)

Heterogeneity: χ^2 = < 0.0001, d.f. =1, P = 1.00

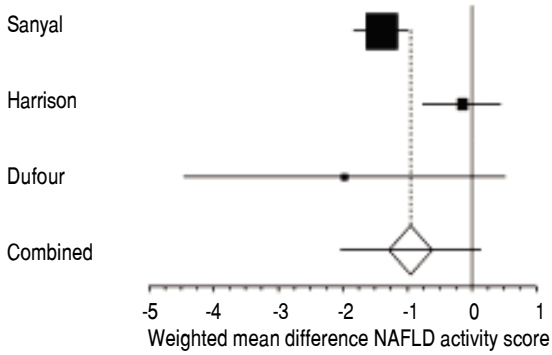
C. Lobular Inflammation



Vitamin E (N)	Placebo (N)	Weighted mean difference (95% CI)
94	94	-0.40 (-0.61 to -0.20)

Heterogeneity: χ^2 = 0.006, d.f. =1, P = 0.94
Test for overall effect Z= -3.86, P = 0.0001

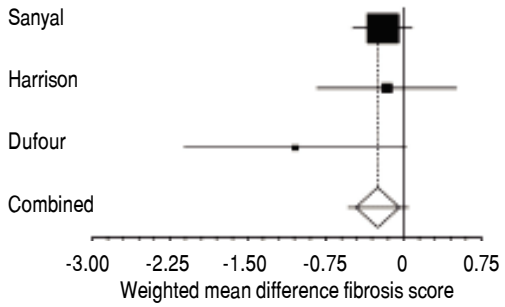
D. NAFLD Activity Score



Vitamin E (N)	Placebo (N)	Weighted mean difference (95% CI)
94	94	-0.95 (-2.04 to 0.13)

Heterogeneity: χ^2 =11.65, d.f. = 2, P = 0.003
Test for overall effect Z = -1.72, P = 0.08

E. Fibrosis



Vitamin E (N)	Placebo (N)	Weighted mean difference (95% CI)
94	94	-0.25 (-0.54 to 0.04)

Heterogeneity: χ^2 = 2.15, d.f. = 2, P = 0.34
Test for overall effect Z = -1.68, P = 0.09

Figure 4. *Vitamin E and histological changes in NAFLD.*

Table 4. Effect of Vitamin E on Biochemical and Anthropometric Variables in NAFLD.

Variable	VIT E (n = 122) Controls (n = 120)	Weighted Means Pre-Treatment	Weighted Means Post-Treatment	P Values Within Group	P value VIT E *vs.* Controls
ALT	VIT E	91.15	69.50	0.001	< 0.0001
	Control	101.80	74.69	0.0001	
BMI	VIT E	34.51	33.36	0.10	0.12
	Control	30.91	29.43	0.004	

ALT: Alanine aminotransferase. BMI: Body mass index.

in and TZDs both showed a significant improvement in fasting blood sugar, HOMA-IR and total cholesterol. Despite improvement in histology with TZD therapy BMI and body weight significantly worsened with treatment. Vitamin E therapy did not significantly alter BMI.

Lifestyle modification including an intense exercise program has shown that a > 7% weight loss is associated with significant improvement in steatosis, necrosis, and inflammation but not fibrosis.[22] However, our meta-analysis reveals that histological response may not necessarily correlate with weight loss given the adverse effects of TZD therapy on body weight. The importance of weight loss has been challenged and a study examining an exercise program without dietary modification revealed liver fat content could decrease without a significant change in body weight.[23,24] As such, our analysis provides further evidence that the pathophysiology involved in the development and progression of NASH may be much more complicated than simply the development of insulin resistance.

Our study also illustrates the limited data and number of RCTs that include histological outcomes in adult patients for the treatment of NASH. In addition, the conclusions arrived by the current studies are limited due to the number of patients involved with only the Sanyal, *et al.* study including more than one hundred patients. Also, pharmaceutical agents studied for efficacy in NAFLD are focused on liver histologic outcomes and do not use cardiovascular disease, malignancy, or mortality as end points although these are important in patients with NAFLD. Though adverse effects are not studied as primary end points and thus, are not easily evaluated in a meta-analysis, all agents studied have been associated with adverse effects which need to be further elucidated prior to implementation. The use of metformin has been associated with the development of lactic acidosis, TZDs may cause an increased incidence of congestive heart failure,[25] and long-term vitamin E may increase risk of prostate cancer.[26] It is unknown whether the changes or improvement seen in histology reverses after cessation of therapy or even translates to an improvement in liver related mortality. In addition, only five trials studied included a diabetic population with the largest trial including non-diabetic subjects. As such, evidenced based guidelines regarding therapy are challenging to construct for the current and future patient population given the increasing incidence of diabetes.

We found that insulin sensitizers and vitamin E play a major role in improving biochemical parameters and over the short term can have a beneficial effect on histologic markers of liver injury. However, it is unknown whether these improvements predict improved clinical outcomes. Future trials should include histological outcomes with longer duration of treatment and follow-up to determine whether or not our current proposed theories of the treatment of NASH are validated. In addition, there may be value in examining if a combination of insulin sensitizers and anti-oxidants provide a synergistic response on histology.

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