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Antioxidant supplements for non-alcoholic fatty liver disease and/or steatohepatitis (Review)

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[Intervention Review]

Antioxidant supplements for non-alcoholic fatty liver disease and/or steatohepatitis

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ABSTRACT

Background

Non-alcoholic fatty liver disease (NAFLD) is characterised by fatty deposition in the hepatocytes of patients with minimal or no alcohol intake and without other known cause. NAFLD includes a wide spectrum of histologic abnormalities ranging from hepatic steatosis to non-alcoholic steatohepatitis (NASH), or even cirrhosis. Antioxidant supplements, therefore, could potentially protect cellular structures against oxidative stress and the resulting lipid peroxidation.

Objectives

To systematically evaluate the beneficial and harmful effects of antioxidant supplements versus no intervention, placebo, or other interventions for patients with NAFLD or NASH.

Search methods

We searched *The Cochrane Hepato-Biliary Group Controlled Trials Register* (June 2006), the *Cochrane Central Register of Controlled Trials* (*CENTRAL*) in *The Cochrane Library* (Issue 2, 2006), *MEDLINE* (1966 to June 2006), *EMBASE* (1980 to June 2006), and *The Chinese Biomedical Database* (1978 to June 2006). No language restrictions were applied.

Selection criteria

Randomised clinical trials evaluating any antioxidant supplements versus no intervention, placebo, or other interventions in patients with NAFLD or NASH. Our inclusion criteria for NAFLD or NASH were based on history of minimal or no alcohol intake, imaging techniques showing hepatic steatosis, and/or histological evidence of hepatic damage (including simple steatosis, fatty infiltration plus nonspecific inflammation, steatohepatitis, fibrosis, and cirrhosis), and by exclusion of other causes of hepatic steatosis.

Data collection and analysis

We extracted data from the identified trials and contacted authors. We used a random-effects model and fixed-effect model with the significant level set at P = 0.05. We evaluated the methodological quality of the randomised trials by looking at how the generation of allocation sequence, allocation concealment, blinding, and follow-up were performed. We made our analyses following the intention-to-treat method by imputing missing data.

Main results

We identified six trials: two were regarded of high methodological quality and four of low methodological quality. None of the trials reported any deaths. Treatment with antioxidant supplements showed a significant, though not clinically relevant, amelioration of aspartate aminotransferase levels, but not of alanine aminotransferase levels, as compared to placebo or other interventions. Gamma-glutamyl-transpeptidase was decreased, albeit not significantly, in the treatment arm. Radiological and histological data were too limited to draw any definite conclusions on the effectiveness of these agents. Adverse events were non-specific and of no major clinical relevance.

Authors' conclusions

There is insufficient data to either support or refute the use of antioxidant supplements for patients with NAFLD. It may be advisable to carry out large prospective randomised clinical trials on this topic.

PLAIN LANGUAGE SUMMARY

No evidence to support or refute antioxidant supplements for patients with non-alcoholic fatty liver disease and/or steatohepatitis

Non-alcoholic fatty liver disease is characterised by fatty deposition in the hepatocytes in the absence of excessive alcohol intake and of other known causes of fatty liver. Hepatic injury might be improved by antioxidant supplements. This systematic review identified six randomised clinical trials. No liver-related or unrelated deaths occurred in any of the included trials. Adverse events were minor and non-specific. Treatment with antioxidant supplements showed a significant, though not clinically relevant, amelioration of aspartate aminotransferase, but not of alanine aminotransferase, as compared to placebo or other interventions. Data on the radiological and/or histological response were too limited to draw any conclusions. Further placebo-controlled trials are necessary.

BACKGROUND

Non-alcoholic fatty liver disease (NAFLD) is characterised by fatty deposition in the liver cells in patients without excessive alcohol intake. Its prevalence is increasing in the developed world, being now recognised as the most common liver disease in the United States and spreading rapidly to the Asia-Pacific region (McCullough 2002; Farrell 2003). In the general population, its estimated prevalence ranges from 3% to 24% (McCullough 2002, Clark 2006). Recent studies show that its prevalence may be as high as 29% in healthy Japanese adults (Jimba 2005) and up to 33% in Americans (Farrell 2006). NAFLD includes a wide spectrum of histologic abnormalities ranging from hepatic steatosis, which has a benign clinical course, to nonalcoholic steatohepatitis (NASH) that may progress to cirrhosis, liver failure, and liver-related death in a significant percentage of patients (McCullough 2002; Farrell 2006; Liou 2006). Indeed, a number of studies suggest that NAFLD may be the cause of cryptogenic cirrhosis (Brolin 1998; Caldwell 1999; Poonawala 2000; Farrell 2003; Liou 2006). Moreover, it remains unclear why only 15% to 20% of patients with NAFLD develop NASH (McCullough 2002).

NAFLD represents the hepatic manifestation of the insulin resistance (or metabolic) syndrome and is particularly associated with obesity, type 2 diabetes mellitus, high triglyceride levels, and low high-density lipoprotein cholesterol levels (Angulo 2002a; Chitturi 2002a; Younossi 2002; Clark 2006). In addition, NAFLD is extremely common in severely obese patients undergoing bariatric surgery, ranging from 84% to 96% (Clark 2006). NAFLD may also occur in children and normal weight people with normal glucose and lipid metabolism (Bacon 1994).

The pathophysiology of NAFLD is not clear. According to the 'two-hits' hypothesis, the first hit involves accumulation of excess fat in the liver cells due to insulin resistance and leads to hepatic steatosis. The second hit concerns oxidative stress that causes lipid peroxidation and activates inflammatory cytokines resulting in NASH (Chitturi 2001; McCullough 2002). More precisely, oxidative stress appears to be the result of an imbalance between pro-oxidant and antioxidant processes in the liver. Such imbalance is likely to be the consequence of: (1) induction of the microsomal cytochrome P450 2E1, which is overexpressed in steatohepatitis because of impaired insulin signalling, (2) mitochondrial release of reactive oxygen species, (3) H_2O_2 production from peroxisomal β-oxidation of fatty acids, and (4) cytokine released from activated inflammatory cells (Younossi 2002). Thus, genes for pro-oxidant (ie, CYP2E1 polymorphism) and antioxidant pathways could also have a role in susceptibility to NASH (Farrell 2003). In addition, these oxidative processes induce a depletion of the potent antioxidants glutathione and vitamin E. The latter is believed to be the 'last antioxidant defence' in lipid membranes. Antioxidant supplements, therefore, could potentially protect cellular structures against damage from oxygen-free radicals and reactive products of lipid peroxidation.

Since NAFLD is usually associated to a number of insulin resistance related conditions, treatment of comorbidity has been regarded of paramount importance in the management of these patients (Angulo 2002b). However, the benefits of this approach have been inconsistent. Indeed, there is no universal treatment for NAFLD/NASH and the pathogenesis remains poorly understood. So far, therapeutic strategies have been largely empirical and experimental trials have mostly been carried out in uncontrolled settings with small sample sizes. Specific therapeutic interventions include weight reduction, ursodeoxycholic acid, clofibrate, gemfibrozil, atorvastatin, troglitazone, and a number of antioxidants such as vitamin E, betaine, and N-acetylcysteine (Angulo 2002b; Younossi 2002; Wang 2002; Neuschwander-T 2003).

We could not find any systematic reviews or meta-analyses addressing the effects of antioxidant supplements for patients with NAFLD or NASH.

OBJECTIVES

To assess beneficial and harmful effects of antioxidants for non-alcoholic fatty liver disease and/or steatohepatitis.

METHODS

Criteria for considering studies for this review

Types of studies

All randomised clinical trials, regardless of publication status, and year, number of patients randomised, language, or blinding.

Types of participants

Participants of any age, sex, or ethnic origin with NAFLD, including NASH and/or cryptogenic cirrhosis diagnosed on the basis of the following criteria:
(1) Imaging techniques showing evidence of hepatic steatosis or steatofibrosis.
(2) Minimal alcohol intake: preferably a daily alcohol intake less than 20 g in women and 40 g in men (Becker 1996; Neuschwander-T 2003).
(3) Liver biopsy evidence (when available) of histologic damage including simple steatosis, fatty infiltration plus nonspecific inflammation, steatohepatitis, fibrosis, and cirrhosis (Brunt 1999; Kleiner 2005).

We excluded trials enrolling patients with other causes of hepatic steatosis or steatofibrosis, including hepatitis B, hepatitis C, autoimmune hepatitis, and genetic liver disease such as Wilson's disease and haemochromatosis. Studies considering patients with one or more causes commonly associated with secondary NAFLD (drugs, surgical procedures, and miscellaneous disorders such as a- or hypo-betalipoproteinaemia, partial lipodystrophy, environmental toxins, or total parenteral nutrition) were also excluded.

Types of interventions

Antioxidants (vitamin A, carotenoids, vitamin C, vitamin E, selenium, and other administered antioxidants that we could identify) at any dose, duration, and route of administration, given separately or in combination versus no intervention, placebo, or other interventions (for example, regimens including a reduction of calories intake, increase in physical activity, behaviour modification, or various surgical interventions aiming at reducing weight). Co-interventions were also considered when used equally in both intervention arms.

Types of outcome measures

Primary outcome measures

(1) All-cause mortality: number of deaths irrespective of cause.

(2) Hepatic-related mortality.
(3) Radiological response (degree of fatty liver infiltration assessed by ultrasound, computer tomography scanning, nuclear magnetic resonance or other imaging techniques).
(4) Biochemical response (serum activities of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatases, gamma-glutamyl-transpeptidase, serum total bilirubin, and ferritin).
(5) Histological response (number of patients with histological improvement/deterioration and changes in the degree of fatty liver infiltration, inflammation, and fibrosis).

Secondary outcome measures
(6) Adverse events (any adverse events as reported in trials). Depending on availability of data, we attempted to classify adverse events as serious or non-serious. Serious adverse events were defined as any outward medical occurrence that was life threatening, resulted in death, or persistent or significant disability, or any medical event, which may have jeopardised the patient or required intervention to prevent it. All other adverse events were considered non-serious.
(7) Quality of life measures.
(8) Cost-effectiveness.

Search methods for identification of studies

We identified relevant randomised clinical trials by searching *The Cochrane Hepato-Biliary Group Controlled Trials Register* (June 2006), the *Cochrane Central Register of Controlled Trials* (*CENTRAL*) in *The Cochrane Library* (Issue 2, 2006), *MEDLINE* (January 1966 to June 2006), and *EMBASE* (January 1980 to June 2006). In addition, we also searched *The Chinese Biomedical Database* (1978 to June 2006). See Appendix 1 for the search strategies we applied to the individual databases.

Data collection and analysis

We followed the instructions given in the *Cochrane Handbook for Systematic Reviews of Intervention* (Higgins 2006) and the *Cochrane Hepato-Biliary Group Module* (Gluud 2006).

Trial selection
Two authors (FL and SO) independently assessed whether the identified trials fulfilled the inclusion criteria. Excluded trials were listed in "Characteristics of excluded studies" with the reasons for exclusions.

Data extraction
SO extracted data, and FL validated the data extraction. We also wrote to authors of included trials asking them to specify the data of interest, which were not clearly reported in their publications.

- Trial characteristics: date, location and funding of the trial, length of follow-up, use of intention-to-treat analyses, as well as the publication status.
- Patient characteristics: number of patients randomised, inclusion and exclusion criteria, mean (or median) age, sex ratio.
- Intervention characteristics: dose, duration and mode of administration of various antioxidants and/or of additional intervention(s).
- Outcome measures: number of events in the intervention group and in the control group (including the number and type of adverse events).

Methodological quality
Due to the risk of overestimation of intervention effects in randomised clinical trials with inadequate methodology (Schulz 1995; Moher 1998; Kjaergard 2001), we assessed the influence of methodological quality using the following components:

Generation of the allocation sequence

- Adequate, if the allocation sequence was generated by a computer or random number table. Drawing of lots, tossing of a coin, shuffling of cards, or throwing dice will be considered as adequate if a person who was not otherwise involved in the recruitment of participants performed the procedure.
- Unclear, if the trial was described as randomised, but the method used for the allocation sequence generation was not described.
- Inadequate, if a system involving dates, names, or admittance numbers were used for the allocation of patients. These studies are known as quasi-randomised and were excluded from the present review when assessing beneficial effects.

Allocation concealment

- Adequate, if the allocation of patients involved a central independent unit, on-site locked computer, identically appearing numbered drug bottles or containers prepared by an independent pharmacist or investigator, or sealed envelopes.
- Unclear, if the trial was described as randomised, but the method used to conceal the allocation was not described.
- Inadequate, if the allocation sequence was known to the investigators who assigned participants or if the study was quasi-randomised.

Blinding

- Adequate, if the trial was described as double blind and the method of blinding involved identical placebo or active drugs.
- Unclear, if the trial was described as double blind, but the method of blinding was not described.
- Not performed, if the trial was not double blind.

Follow-up

- Adequate, if the numbers and reasons for dropouts and withdrawals in all intervention groups were described or if it was specified that there were no dropouts or withdrawals.
- Unclear, if the report gave the impression that there had been no dropouts or withdrawals, but this was not specifically stated.
- Inadequate, if the number or reasons for dropouts and withdrawals were not described.

Statistical methods
We performed the meta-analyses according to the recommendations of The Cochrane Collaboration (Higgins 2006). We used the software package RevMan 4.2 (RevMan 2003) provided by The Cochrane Collaboration. We presented dichotomous variables as risk difference (RD) or odds ratios (OR) with 95% confidence interval (CI) and continuous outcome measures as weighted mean differences (WMD) with 95% CI. The fixed-effect model (DeMets 1987) as well as the random-effects model were used, with the significant level set at P = 0.05. The analyses included all patients irrespective of compliance or follow-up following the

'intention-to-treat' principle and using the last reported observed response ('carry forward'). In addition, subgroup analyses were performed according to the methodological quality of the trials. We planned to use funnel plot asymmetry to assess the existence of publication bias and other biases (Egger 1997).

RESULTS

Description of studies

Search results

In total we identified 106 references: 81 through *PUBMED* and *EMBASE*, 2 through *The Cochrane Hepato-Biliary Group Controlled Trials Register*, and 23 through the *Cochrane Central Register of Controlled Trials* in *The Cochrane Library* (thirteen of these were not present in *PUBMED*, but none was relevant). Of the 81 references identified by *PUBMED* and *EMBASE*, 10 were clearly irrelevant to this review and were excluded on the basis of their titles and abstracts. Of the remaining 71 references, 13 were studies of antioxidants usage for non-alcoholic fatty liver disease or steatohepatitis. Another randomised trial was an ongoing trial published in an abstract form. Thus, a total of fifteen completed trials were identified: of these, six were selected for inclusion, whereas nine were excluded from the review.

Included trials

Six randomised clinical trials of antioxidants usage met the criteria for this review. All were published as full text articles. The included trials differed markedly in their inclusion criteria: two (Miglio 2000; Vajro 2004) assessed hepatic steatosis through ultrasonographic investigation, whereas the remaining four trials (Rui 2001; Harrison 2003; Pamuk 2003; Bugianesi 2005) diagnosed NAFLD/NASH by means of histology. Moreover, in one trial (Vajro 2004), the included patients were children with a mean age of 10 years, whereas the remaining trials enrolled adult patients (age > 18 years). In addition, in four trials (Harrison 2003; Pamuk 2003; Vajro 2004; Bugianesi 2005) hypertransaminasaemia, ie, increased alanine aminotransferase (ALT) and aspartate aminotransferase (AST) serum levels or increased ALT serum levels only, was the main inclusion criteria (see Table of included studies). Major exclusion criteria were: other causes of chronic liver disease (hepatitis B and/or C, haemochromatosis, alpha1-antitrypsin deficiency, Wilson's disease, autoimmune liver disease), use of drugs associated with the development of NASH, and alcohol abuse. The latter was defined as alcohol consumption exceeding respectively 10 g/day, 30 g/day and 20 g/day (Harrison 2003; Pamuk 2003; Bugianesi 2005). In half of the trials it was not reported.

The type of intervention in the experimental arm varied greatly among the different trials. Three trials (Harrison 2003; Vajro 2004, Bugianesi 2005) used vitamin E in different formulations and dosages, either alone or in combination with other antioxidants. In one trial (Miglio 2000), a combination of betaine glucuronate, diethanolamine glucuronate, and nicotinamide ascorbate was used. In another (Pamuk 2003), n-acetylcysteine was administered. Finally, in the Chinese trial (Rui 2001) reduced glutathione was administered intravenously. The duration of treatment ranged from two weeks (Rui 2001) to twelve months (Bugianesi 2005).

The intervention in the control arm was placebo or no treatment in four trials (Miglio 2000; Harrison 2003, Pamuk 2003; Vajro 2004), a mixture of herbs called DanNingPian administered orally in the Chinese trial (Rui 2001), and metformin in the remaining one (Bugianesi 2005).

Additional interventions offered to both intervention arms included a low-calorie diet and a moderate daily exercise programme in two trials (Harrison 2003; Vajro 2004).

The sample size varied greatly among the included trials: from 28 (Vajro 2004) to 191 (Miglio 2000) patients.

Excluded studies

A total of nine studies were excluded (*see* Table of excluded studies). Six of these studies were not randomised; moreover, the open-label pilot study by Lavine (Lavine 2000) was excluded also because NASH diagnosis was erroneously made upon ultrasonographic findings and not upon histological features. The study by Sanyal et al (Sanyal 2004) was excluded because it was a comparative study assessing the efficacy of vitamin E alone versus a combination of vitamin E and pioglitazone. In the study by Lu (Lu 2005), there was no control arm, but only a comparison between two different dosages of the same agent. Finally, in the trial by Kugelmans (Kugelmas 2003), data were presented before and after therapy and not based on patients who received or did not receive vitamin E.

Ongoing trials

A two-year multicentre randomised placebo-controlled trial by Dufour et al (Dufour 2005), published in abstract form, is an ongoing trial assessing the efficacy of ursodeoxycholic acid in combination with vitamin E to treat NASH.

Risk of bias in included studies

None of the included trials reported power calculations to assess sample size.

Allocation sequence was considered adequate in two trials (Harrison 2003; Bugianesi 2005): it was computer-generated in the former and based on a random sequence in the latter. In the remaining four trials (Miglio 2000; Rui 2001; Pamuk 2003; Vajro 2004) it was either not described or unclear.

Allocation concealment was considered adequate in three trials (Harrison 2003; Vajro 2004; Bugianesi 2005): it was performed by the pharmacy in the first one and by means of sealed envelopes in the other two. In the remaining three trials (Miglio 2000; Rui 2001; Pamuk 2003) it was either not described or unclear.

Blinding was considered adequate in three trials (Miglio 2000; Harrison 2003; Vajro 2004), whereas it was either not performed or unclear in the remaining three (Rui 2001; Pamuk 2003; Bugianesi 2005).

Follow-up was considered adequate in all the included trials except the Chinese trial (Rui 2001), in which it was unclear.

The trials with all four components adequate, ie, generation of the allocation sequence, allocation concealment, blinding and follow-up, were regarded as trials of high-methodological quality (Miglio 2000; Harrison 2003). The remaining trials, with one or more unclear or inadequate quality components, were classified as low methodological quality and, therefore, regarded as high-bias risk trials (Rui 2001; Harrison 2003; Vajro 2004; Bugianesi 2005).

Effects of interventions

All-cause mortality and liver-related mortality

None of the included trials reported any fatalities related or unrelated to liver disease.

Radiological response

Radiological response was assessed by means of ultrasound in two trials (Miglio 2000; Vajro 2004). In the trial by Miglio et al, ultrasonography (US) steatosis score was reduced by 0.49 (SD = 0.63) points in the antioxidant group, whereas it remained virtually unchanged in the control group (-0.05, SD = 0.66). WMD was -0.44 IU/L (95% CI -0.63 to -0.25). In the trial by Vajro et al, bright liver on US persisted in 11/14 patients in the antioxidant group and in 8/14 in the control arm. The odds ratio was 2.75 (95% CI 0.52 to 14.44).

Biochemical response

Biochemical response was assessed by measuring the serum activities of aspartate aminotransferase, alanine aminotransferase, gamma-glutamyltranspeptidase, and serum total bilirubin levels.

Aspartate aminotransferase (AST) activity was evaluated in only one high-methodological quality trial (Miglio 2000) and in two low-methodological quality ones (Rui 2001; Pamuk 2003). Overall, antioxidants-treated patients showed only a slight, but significant (P = 0.004) decrease in AST activity (WMD -5.28 IU/L, 95% CI -8.84 to -1.72) compared to placebo-treated subjects.

Alanine aminotransferase (ALT) activity was evaluated in all six trials. In the high-quality group WMD was -26.77 IU/L (95% CI -32.76 to -20.78), whereas in the low-quality group WMD was 34.82 IU/L (95% CI 31.00 to 38.64). Overall, antioxidants significantly increased ALT activity (P < 0.00001): WMD was 17.01 IU/L (95% CI 13.79 to 20.23). In order to clarify whether the type of intervention could have affected the results, we further divided the trials into two sub-groups (vitamin E compared to other antioxidants) and assessed again the effect on ALT activity (Comparison 02-03). The vitamin E interventions were associated with a significant increase in ALT activity (Harrison 2003; Vajro 2004; Bugianesi 2005), whereas the other types of antioxidants seemed to significantly decrease ALT activity (Miglio 2000; Rui 2001; Pamuk 2003).

Gamma-glutamyl-transpeptidase (GGT) activity was evaluated in one high-quality trial (Miglio 2000) and in two low-quality ones (Rui 2001; Pamuk 2003). In both sub-groups, GGT activity seemed to decrease slightly, but not significantly (WMD -8.60 IU/L, 95% CI -25.41 to 8.21) and (WMD -6.84 IU/L , 95% CI -29.59 to 15.90), respectively.

Serum total bilirubin levels were assessed only in two low-quality trials (Rui 2001; Pamuk 2003), showing virtually no change (WMD 0.10 IU/L, 95% CI -0.22 to 0.42).

No trials investigated changes in ALP and ferritin levels following the administration of antioxidant supplements, placebo, or in respect to other interventions.

Histological response

Histological response was assessed only in the trial by Harrison et al (Harrison 2003) who evaluated the scores of inflammation/necrosis and fibrosis before and after treatment. The combined inflammation/necrosis score did not change in the placebo or vitamin group with time. In 11/23 patients in the antioxidant group and in 9/22 patients in the control group there was an improvement in the fibrosis score (OR = 0.76, 95% CI 0.23 to 2.46), by a mean of -0.50 (SD = 1.00) and -0.25 points (SD = 1.25), respectively (WMD -0.25 IU/L, 95% CI -0.91 to 0.41). However, these results were not significantly different. In the trial by Bugianesi et al (Bugianesi 2005), a post-treatment biopsy was performed only in 17/55 metformin-treated patients, whereas none of the patients included in the vitamin E group underwent a second liver biopsy.

Adverse events

Among the six trials, four provided information on adverse events. In the trials by Harrison et al (Harrison 2003) and Bugianesi et al (Bugianesi 2005) no adverse events were reported. Vajro et al (Vajro 2004) reported an increase in serum transaminase after starting the administration of vitamin E in one patient. In the trial by Miglio (Miglio 2000) there were 10 cases of adverse events among 96 patients in the antioxidant group and 6 cases among 95 patients in the control group: all adverse events were not serious and included mild to moderate headache, nausea, diarrhoea, heartburn, and meteorism. In the trial by Pamuk (Pamuk 2003) no information about adverse events was provided, and in the Chinese trial (Rui 2001) this issue could not be evaluated.

Quality-of-life measures and cost effectiveness

Quality of life measures and cost effectiveness were not reported in any of the trials.

Bias exploration

We were unable to perform the funnel plot analysis as stated in the protocol, as both visual examination and statistical analysis of funnel plots have limited power to detect bias if the number of trials is small. We, therefore, could not assess the existence of bias by funnel plot.

DISCUSSION

We identified six randomised clinical trials assessing the effects of antioxidant supplements for patients with NAFLD and NASH. There was a considerable heterogeneity among these trials in respect to inclusion criteria, sample size, age (adults and children), type of interventions, type of control interventions, duration of treatment, and methods of outcome assessment. It could very well be argued that we should have excluded the Bugianesi 2005 et al trial as they administered metformin in the control group. We decided to keep it included because there is no evidence that metformin significantly affects prognosis in non-alcoholic fatty liver disease and/or non-alcoholic steatohepatitis (Angelico 2007) and the exclusion would not dramatically have an effect on our results. Moreover, the included trials varied substantially regarding methodological quality and hence bias risk.

Based on allocation sequence generation, allocation concealment, blinding, and follow-up, two trials were regarded as being of high methodological quality (Miglio 2000; Harrison 2003). Of these, one (Harrison 2003) had a small sample size (45 patients), whereas the trial by Miglio et al (Miglio 2000) included a total of 191 patients. The remaining four trials (Rui 2001; Pamuk 2003; Vajro 2004; Bugianesi 2005) resulted mostly as inadequate or unclear in at least one of the four components used to assess methodological quality.

No fatalities were recorded in any of the six trials. This was partly due to the fact that all patients enrolled in all, but one (Bugianesi 2005) trial, did not have an advanced liver disease. Also, the duration of therapy with a maximum of twelve months was not

long enough to detect long-term complications of the disease, such as decompensation, hepatocellular carcinoma, or death. Indeed, the few cases of cirrhosis included in the trials were diagnosed by means of liver biopsy and not on clinical grounds, suggesting that these patients were in a stable and compensated condition.

Biochemical response was evaluated by means of the serum activities of different hepatic enzymes and total bilirubin. Three trials assessed the effect of antioxidant supplements on aspartate aminotransferase (AST) activity; only one of these being regarded of high methodological quality. Overall, they showed a slight, but significant reduction of AST levels in the experimental arm. Nevertheless, the high degree of heterogeneity measured with the I^2 test makes the interpretation of these findings difficult. Serum alanine aminotransferase (ALT) activity was the main outcome measured in all the six trials. The pattern of results for ALT activity in the low-methodological quality sub-group showed a significant beneficial effect in the control arm. However, this could have been biased by the large standard deviation observed in one trial (Rui 2001) and, again, by the high degree of heterogeneity (I^2 = 86.4%) observed among the four low-methodological quality trials. In the high-methodological quality sub-group, the analysis seemed to favour the treatment arm, although heterogeneity was high (I^2 = 87.0). Overall, the analysis showed a beneficial effect in the control arm, although there was a high degree of heterogeneity (I^2 = 98.4%). Thus, the beneficial effect of antioxidant supplements on hepatic cytolysis cannot be evaluated on the basis of the identified trials. As regards the effect of antioxidant supplements on the indices of cholestasis, the pattern of results for GGT was similar in both the high- and the low-methodological quality trials (Miglio 2000; Rui 2001; Pamuk 2003); a slight, but not significant decrease of the activity of this enzyme in the experimental arm was observed. Serum total bilirubin remained virtually unchanged amongst the two trials evaluating this marker (Rui 2001; Pamuk 2003).

As regards the radiological response, this was assessed in only two trials (Miglio 2000; Vajro 2004) with contradictory results. In the first trial (Miglio 2000), carried out in adults, an eight-week treatment with betaine glucuronate, combined with diethanolamine glucuronate and nicotinamide ascorbate, caused a 25% reduction of hepatic steatosis evaluated by US scanning, which resulted highly significant. By contrast, the trial by Vajro et al (Vajro 2004), which was carried out in 28 children with obesity-related hypertransaminasaemia, treated for five months with either vitamin E or placebo, showed that disappearance of the bright liver occurred only in patients who lost weight and was twice as common in the children taking the placebo. Therefore, no conclusion can be drawn on the efficacy of antioxidant supplements in respect to changes of US hepatic steatosis, due to the limited number of trials, the different age of the participants, the type of interventions, the duration of treatment, and the sample size.

As to the histological response, only Harrison et al (Harrison 2003) included this outcome measure in their trial. Although there was a trend showing a beneficial effect on hepatic fibrosis (both in terms of number of patients with less fibrosis and improvement of the fibrosis score), there is not enough data to adequately examine the effect of antioxidant supplements on this histological feature. Besides, there was no significant effect of vitamins E and C on hepatic inflammation and necrosis.

Finally, if one considers the type of intervention used in the different trials, antioxidant supplements other than vitamin E seem to exert a beneficial effect on the activity of ALT (Comparison 02-03) (Miglio 2000; Rui 2001; Pamuk 2003) as well as on US steatosis (Miglio 2000). The concept that different antioxidants can exhibit different effects on liver structure and function is emphasised. However, these findings need to be confirmed by further randomised clinical trials, comparing specifically the effect of vitamin E versus other antioxidants. A further issue for discussion relates to the dose of vitamin E administered in the trials by Harrison et al (Harrison 2003), Bugianesi et al (Bugianesi 2005), and Vajro et al (Vajro 2004). In all three trials a daily dose of vitamin E ranging from 600 IU to 1000 IU was used, either alone on in combination with vitamin C. A major recent meta-analysis study (Miller 2005) assessed the risk of high dosage of vitamin E supplementation in 19 clinical trials for a total of 136,000 patients. The dosage of vitamin E ranged from 16.5 IU/day to 2000 IU/day (median 400 IU/day). The dose response analysis showed a statistically significant relationship between vitamin E dosage and all-cause mortality, with an increased risk of all-cause mortality for dosages greater than 400 IU/day. It is, therefore, not possible that the dosages of vitamin E may have been too high in the assessed trials (Harrison 2003; Vajro 2004; Bugianesi 2005), having caused the increased serum ALT activities.

No trials used validated assessments of symptoms. We performed an adverse events analysis. Harrison et al (Harrison 2003) found that no patient experienced any adverse events. Bugianesi et al (Bugianesi 2005) reported that no patient had to discontinue the trial because of adverse events to medication. Vajro et al (Vajro 2004) found that one of their young patients showed an increase in serum transaminase after starting treatment with vitamin E. Miglio et al (Miglio 2000) reported a number of non-specific symptoms (including nausea, diarrhoea, headache, heartburn and meteorism), regarded as non-serious, in 10% of patients of the experimental group and in 6% of the patients of the control group. Considering the individual symptoms, none of these resulted significantly different between the two trial arms. Moreover, it is difficult to assess a cause-effect relationship with the experimental interventions since all reported adverse events are common symptoms in patients with liver disorders.

In conclusion, the results of the randomised trials identified in this review do not allow us to provide evidence pros or cons antioxidant supplements in NAFLD or NASH, due to the small number of trials, their low methodological quality, the variability in clinical and histological assessment, and the number of different tested antioxidant supplements.

AUTHORS' CONCLUSIONS

Implications for practice

There is not enough evidence to recommend or refute antioxidant supplements in patients with NAFLD or NASH. Vitamin E may increase the activity of alanine aminotransferase in these patients.

Implications for research

Further randomised clinical trials are needed if the potential beneficial and harmful effects of antioxidant supplements for NAFLD and NASH is to be evaluated. In such trials, a sound methodology with careful consideration of the inclusion criteria

and of the outcome measures will be necessary. Moreover, it is advisable to design randomised clinical trials with a large enough sample size, including a well-defined type of antioxidant supplements, given for longer periods of time and compared with placebo. Such randomised trials ought to be reported according to the CONSORT guidelines (http://www.consort-statement.org).

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CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Bugianesi 2005

Methods	Generation of allocation sequence: adequate, random sequence. Allocation concealment: adequate, sealed envelopes. Blinding: not performed. Follow-up: adequate.
Participants	Inclusion criteria: - elevation of ALT > 1.5 times normal values for six months or more - histological diagnosis of NAFLD Exclusion criteria: - other causes for chronic liver disease (hepatitis B and C, hereditary hemochromatosis, autoimmune hepatitis) - diabetes mellitus - celieac disease - BMI >= 35 kg/m2 - a history of alcohol consumption greater than 20 g/day. Characteristics of included patients: n = 83 Mean age = 42 Males = 82%
Interventions	Vitamin E (800 IU/day) versus metformin (2 g/day) for 12 months.
Outcomes	Assessment of liver enzymes, HOMA-IR, parameters of the metabolic syndrome at the beginning and at the end of the treatment period. Pre- and post-treatment histology available only in 17/55 patients in the metformin group.
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Allocation concealment?	Low risk	A - Adequate

Harrison 2003

Methods	Generation of allocation sequence: adequate, computer-generated randomisation table. Allocation concealment: adequate, performed by a pharmacy. Blinding: adequate, double blinding. Follow-up: adequate.
Participants	Inclusion criteria: - clinical and histologic diagnosis of NASH - age >18 years - liver biopsy within the past 6 months for elevated aminotransferases - well compensated liver disease (Hb at least 12 g/dl for women and 13 g/dl for men, white blood cell count of greater than 3.000/mm3, neutrophil count of greater than 1500/mm3, platelets greater than 70.000/mm3, serum albumin greater than 1.4 mg/dl and a serum creatinine less than 1.4 mg/dl). Exclusion criteria: - other causes for chronic liver disease (hepatitis B and C, hereditary hemochromatosis, alpha-1 antitrypsin deficiency, Wilson's disease, or autoimmune liver disease) - use of drugs associated with the development of steatohepatitis - prior surgical procedures - evidence of decompensated liver disease, such as a history of ascites, bleeding varices, hepatic encephalopathy - pregnancy - total parental nutrition within the past 6 months - a history of organ transplant - other conditions that have been known to cause NASH or worsen the disease - a history of alcohol consumption greater than 10 g/day. Characteristics of included patients: n = 49 Mean age = 51 Males = 44%
Interventions	Vitamin E 1000 IU and Vitamin C 1000 mg per day versus placebo for six months. All patients in both groups were given the same 1600-calorie diet and written exercise plan as outlined by the National Institutes of Health and the National Heart, Lung and Blood Institute.
Outcomes	Biochemical and histological outcomes were assessed before and after treatment.
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Allocation concealment?	Low risk	A - Adequate

Miglio 2000

Methods	Generation of allocation sequence: adequate, computer-generated. Allocation concealment: adequate, sealed envelopes. Blinding: adequate, double blinding.

Miglio 2000 *(Continued)*

	Follow-up: adequate.
Participants	Inclusion criteria: -liver enlargement -ultrasound steatosis -age >18 years. Exclusion criteria: -alcoholic past or present abuse -viral hepatitis, mononucleosis, cytomegalovirus or spirochete infection -Wilson's disease -Crohn's disease -pregnancy or nursing -uncooperative patients or patients not able or not willing to release informed consent Characteristics of included patients: Mean age = 57 Males = 70 %
Interventions	2 capsules/day containing betaine glucuronate 150 mg, diethanolamine glucuronate 30 mg, nicotinamide ascorbate 20 mg versus placebo for eight weeks.
Outcomes	Outcomes assessed at the beginning and at the end of the study were: -complete clinical examination -laboratory investigations -ultrasound response.
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Allocation concealment?	Low risk	A - Adequate

Pamuk 2003

Methods	Generation of allocation sequence: unclear. Allocation concealment: unclear. Blinding: not performed. Follow-up: adequate.
Participants	Inclusion criteria: - biopsy proven NASH - high alanine aminotransferase and/or aspartate aminotransferase levels at least 2 occasions in the past 6 months - adults Exclusion criteria: - daily alcohol intake >30 g - patients with steatohepatitis accompanying other liver diseases, or systemic diseases other than obesity, hyperlipidemia, diabetes, intake of hepatotoxic drugs or lipid-lowering agents Characteristics of included patients: n = 18 Mean age = 49 (SD 10)

Pamuk 2003 *(Continued)*

	Males = 53 %
Interventions	N-acetylcysteine (600 mg/day) for 4 weeks versus no treatment.
Outcomes	Outcomes assessed at the beginning and at the end of the study were: -laboratory investigations -BMI.
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Allocation concealment?	Unclear risk	B - Unclear

Rui 2001

Methods	Generation of allocation sequence: unclear. Allocation concealment: unclear. Blinding: not performed. Follow-up: unclear. Sample size: no.
Participants	Inclusion criteria: - biopsy proven NASH - no history of alcohol abuse - adults. Exclusion criteria: not reported. Characteristics of included patients: n = 22 Mean age = 53.1 (SD 7.9) Males = 41%
Interventions	DanNingPian (5 tablets TID for 12 weeks) versus reduced glutathione (TAD) (1200 mg with 0.9 NS 500 ml IV infusion QD for 2 to 3 weeks).
Outcomes	Liver function tests assessed at 4 and 12 weeks. Definition of remarkably effective: two or more than two liver function indicators completely back to normal. Definition of effective: two or more than two liver function indicators reduced for more than 50%. Definition of ineffective: no improvement or others.
Notes	Also patients with type 2 diabetes/IGT, hyperlipidemia and/or obesity were included in both groups. The majority of them complained of GI symptoms.

Risk of bias

Bias	Authors' judgement	Support for judgement
Allocation concealment?	Unclear risk	B - Unclear

Vajro 2004

Methods	Generation of allocation sequence: unclear. Allocation concealment: adequate, sealed envelopes. Blinding: adequate. Follow-up: adequate.
Participants	Inclusion criteria: - body weight >= 120% according to Tanner - BMI > 95th percentile -hypertransaminasemia (ALT or AST >= 1.5 times above normal values for more than 6 months - US hepatic steatosis - children. Exclusion criteria: - hepatitis B and/or C - haemochromatosis - alpha1-antitrypsin deficiency - Wilson's disease - autoimmune liver disease - use of drugs associated with the development of NASH - history of alcohol abuse - cystic fibrosis - hereditary fructose intolerance - aminoacid disorders - malnutrition - atypical celiac disease. Characteristics of included patients: n = 28 Mean age = 43 Males = 88% in Bologna, 77% in Turin Cirrhotics = 3.6 %
Interventions	Placebo (for 5 months) versus oral alpha-acetate tocopherol (400 mg/day for 2 months, then 100 mg/day for 3 months). All patients received a balanced low-calorie diet and underwent a moderate daily exercise programme.
Outcomes	Outcomes assessed at month 2 and month 5 were: - loss of weight - ALT levels - Vitamin E / cholesterol ratio.
Notes	During the study a reallocation of the patients was made from initial groups between diet and vitamin E compliers and noncompliers.

Risk of bias

Bias	Authors' judgement	Support for judgement
Allocation concealment?	Low risk	A - Adequate

> = more or equal to; greater or equal to.
> more than.

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Abdelmalek 2001	Patients were consecutively, but not randomly, enrolled.
Dentico 1995	The study was not randomised.
Hasegawa 2001	Pilot study. No control group.
Kugelmas 2003	Data for all biochemical variables were presented combining patients who did or did not receive vitamin E.
Lavine 2000	Open-label non-randomised pilot study. NASH diagnosis was made on ultrasound and not on histology.
Lu 2005	There was no control arm, but only a comparison between two different dosages of the same agent.
Merat 2003	The allocation was not randomised.
Sanyal 2004	This study compared the efficacy of vitamin E alone and a combination of vitamin E and pioglitazone.
Trappoliere 2005	The allocation was not randomised.

Characteristics of ongoing studies *[ordered by study ID]*

Dufour 2005

Trial name or title	A 2-years multicenter randomised placebo-controlled study testing UDCA in combination with vitamin E to treat NASH.
Methods	
Participants	48 patients with elevated aminotransferases, drinking less than 40g alcohol/week with liver biopsy-proven NASH less than 6 months prior inclusion and without other liver condition were enrolled.
Interventions	UDCA 12 mg/kg/d to 15 mg/kg/d with vitamin E 2 X 400 IU/d or UDCA with placebo or placebo/placebo for 2 years.
Outcomes	Outcomes assesed at the beginning and at the end of the study were: -liver biopsy -laboratory investigations.
Starting date	2005
Contact information	
Notes	

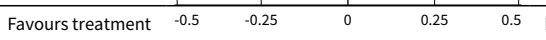
DATA AND ANALYSES

Comparison 1. Mortality

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 All cause mortality	6	429	Risk Difference (M-H, Fixed, 95% CI)	0.0 [-0.02, 0.02]
1.1 High quality	2	236	Risk Difference (M-H, Fixed, 95% CI)	0.0 [-0.02, 0.02]
1.2 Low quality	4	193	Risk Difference (M-H, Fixed, 95% CI)	0.0 [-0.04, 0.04]

Analysis 1.1. Comparison 1 Mortality, Outcome 1 All cause mortality.

Study or subgroup	Antioxidants n/N	Control n/N	Risk Difference M-H, Fixed, 95% CI	Weight	Risk Difference M-H, Fixed, 95% CI
1.1.1 High quality					
Harrison 2003	0/23	0/22		10.71%	0[-0.08,0.08]
Miglio 2000	0/96	0/95		45.48%	0[-0.02,0.02]
Subtotal (95% CI)	**119**	**117**		**56.19%**	**0[-0.02,0.02]**
Total events: 0 (Antioxidants), 0 (Control)					
Heterogeneity: Tau^2=0; Chi^2=0, df=1(P=1); I^2=0%					
Test for overall effect: Not applicable					
1.1.2 Low quality					
Bugianesi 2005	0/28	0/55		17.67%	0[-0.05,0.05]
Pamuk 2003	0/18	0/17		8.33%	0[-0.1,0.1]
Rui 2001	0/22	0/25		11.15%	0[-0.08,0.08]
Vajro 2004	0/14	0/14		6.67%	0[-0.13,0.13]
Subtotal (95% CI)	**82**	**111**		**43.81%**	**0[-0.04,0.04]**
Total events: 0 (Antioxidants), 0 (Control)					
Heterogeneity: Tau^2=0; Chi^2=0, df=3(P=1); I^2=0%					
Test for overall effect: Not applicable					
Total (95% CI)	**201**	**228**		**100%**	**0[-0.02,0.02]**
Total events: 0 (Antioxidants), 0 (Control)					
Heterogeneity: Tau^2=0; Chi^2=0, df=5(P=1); I^2=0%					
Test for overall effect: Not applicable					
Test for subgroup differences: Not applicable					

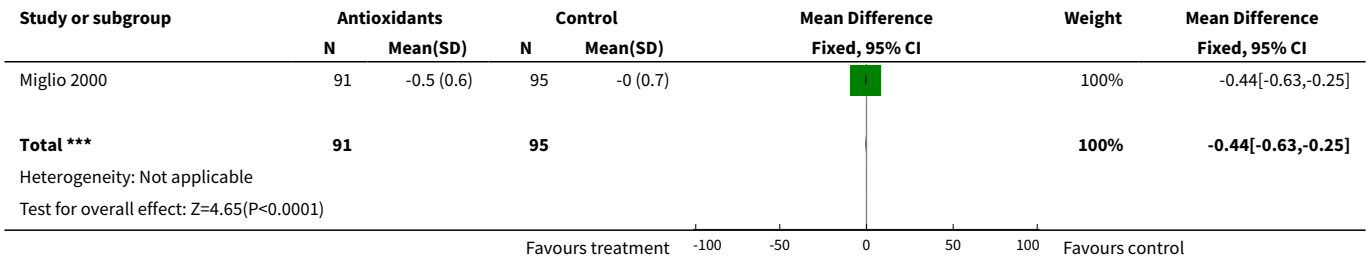


Comparison 2. Radiological response

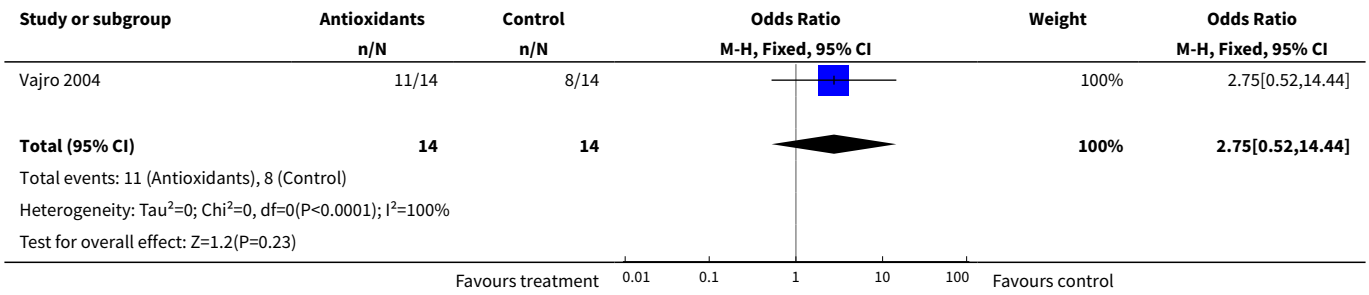
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 US pattern improvement	1	186	Mean Difference (IV, Fixed, 95% CI)	-0.44 [-0.63, -0.25]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
2 US bright liver persistence	1	28	Odds Ratio (M-H, Fixed, 95% CI)	2.75 [0.52, 14.44]

Analysis 2.1. Comparison 2 Radiological response, Outcome 1 US pattern improvement.



Analysis 2.2. Comparison 2 Radiological response, Outcome 2 US bright liver persistence.



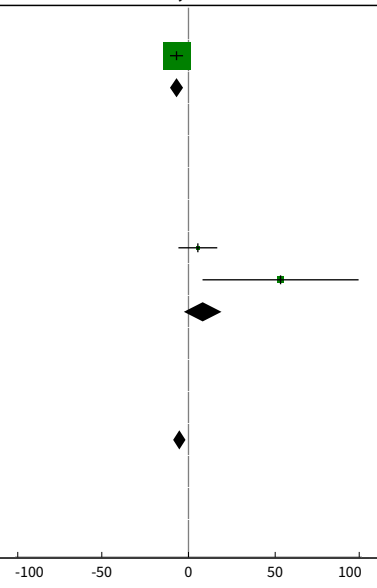
Comparison 3. Biochemical response

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Aspartate aminotransferase activity (IU/L)	3	273	Mean Difference (IV, Fixed, 95% CI)	-5.28 [-8.84, -1.72]
1.1 High quality	1	191	Mean Difference (IV, Fixed, 95% CI)	-6.9 [-10.67, -3.13]
1.2 Low quality	2	82	Mean Difference (IV, Fixed, 95% CI)	8.18 [-2.68, 19.05]
2 Alanine aminotransferase activity (IU/L))	6	429	Mean Difference (IV, Fixed, 95% CI)	17.01 [13.79, 20.23]
2.1 High quality	2	236	Mean Difference (IV, Fixed, 95% CI)	-26.77 [-32.76, -20.78]
2.2 Low quality	4	193	Mean Difference (IV, Fixed, 95% CI)	34.82 [31.00, 38.64]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
3 Alanine aminotransferase activity (Vitamin E vs other antioxidants) (IU/L)	6	429	Mean Difference (IV, Fixed, 95% CI)	17.01 [13.79, 20.23]
3.1 Vitamin E trials	3	156	Mean Difference (IV, Fixed, 95% CI)	35.59 [31.72, 39.46]
3.2 Other antioxidants trials	3	273	Mean Difference (IV, Fixed, 95% CI)	-25.06 [-30.88, -19.24]
4 Gamma-glutamyl-transpeptidase activity (IU/L)	3	273	Mean Difference (IV, Fixed, 95% CI)	-7.98 [-21.50, 5.54]
4.1 High quality	1	191	Mean Difference (IV, Fixed, 95% CI)	-8.6 [-25.41, 8.21]
4.2 Low quality	2	82	Mean Difference (IV, Fixed, 95% CI)	-6.84 [-29.59, 15.90]
5 Serum total bilirubin levels (umol/L)	2	82	Mean Difference (IV, Fixed, 95% CI)	0.10 [-0.22, 0.42]
5.1 High quality	0	0	Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
5.2 Low quality	2	82	Mean Difference (IV, Fixed, 95% CI)	0.10 [-0.22, 0.42]

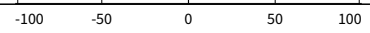
Analysis 3.1. Comparison 3 Biochemical response, Outcome 1 Aspartate aminotransferase activity (IU/L).

Study or subgroup	Antioxidants N	Antioxidants Mean(SD)	Control N	Control Mean(SD)	Mean Difference Fixed, 95% CI	Weight	Mean Difference Fixed, 95% CI
3.1.1 High quality							
Miglio 2000	96	-4.7 (15.3)	95	2.2 (10.9)		89.27%	-6.9[-10.67,-3.13]
Subtotal ***	**96**		**95**			**89.27%**	**-6.9[-10.67,-3.13]**
Heterogeneity: Not applicable							
Test for overall effect: Z=3.59(P=0)							
3.1.2 Low quality							
Pamuk 2003	18	-4.6 (15)	17	-10 (18.5)		10.11%	5.4[-5.8,16.6]
Rui 2001	22	-12.7 (91.8)	25	-65.8 (60)		0.63%	53.1[8.1,98.1]
Subtotal ***	**40**		**42**			**10.73%**	**8.18[-2.68,19.05]**
Heterogeneity: Tau^2=0; Chi^2=4.07, df=1(P=0.04); I^2=75.4%							
Test for overall effect: Z=1.48(P=0.14)							
Total ***	**136**		**137**			**100%**	**-5.28[-8.84,-1.72]**
Heterogeneity: Tau^2=0; Chi^2=10.67, df=2(P=0); I^2=81.26%							
Test for overall effect: Z=2.91(P=0)							
Test for subgroup differences: Chi^2=6.61, df=1 (P=0.01), I^2=84.86%							



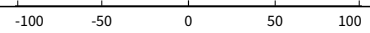
Analysis 3.2. Comparison 3 Biochemical response, Outcome 2 Alanine aminotransferase activity (IU/L)).

Study or subgroup	Antioxidants N	Antioxidants Mean(SD)	Control N	Control Mean(SD)	Mean Difference Fixed, 95% CI	Weight	Mean Difference Fixed, 95% CI
3.2.1 High quality							
Harrison 2003	23	-10 (60)	22	-30 (55)		0.92%	20[-13.61,53.61]
Miglio 2000	96	-4.7 (19.3)	95	23.6 (23.4)		28%	-28.3[-34.39,-22.21]
Subtotal ***	**119**		**117**			**28.92%**	**-26.77[-32.76,-20.78]**
Heterogeneity: Tau²=0; Chi²=7.68, df=1(P=0.01); I²=86.98%							
Test for overall effect: Z=8.76(P<0.0001)							
3.2.2 Low quality							
Bugianesi 2005	28	-13 (8)	55	-50 (10)		65.81%	37[33.03,40.97]
Pamuk 2003	18	-18.2 (24.5)	17	-15 (40)		2.12%	-3.2[-25.33,18.93]
Rui 2001	22	-3.3 (90.1)	25	-64.4 (62.3)		0.52%	61.1[16.22,105.98]
Vajro 2004	14	-24.1 (20.7)	14	-29.9 (31.7)		2.64%	5.83[-14,25.66]
Subtotal ***	**82**		**111**			**71.08%**	**34.82[31,38.64]**
Heterogeneity: Tau²=0; Chi²=22.02, df=3(P<0.0001); I²=86.38%							
Test for overall effect: Z=17.86(P<0.0001)							
Total ***	**201**		**228**			**100%**	**17.01[13.79,20.23]**
Heterogeneity: Tau²=0; Chi²=318.41, df=5(P<0.0001); I²=98.43%							
Test for overall effect: Z=10.35(P<0.0001)							
Test for subgroup differences: Chi²=288.71, df=1 (P<0.0001), I²=99.65%							



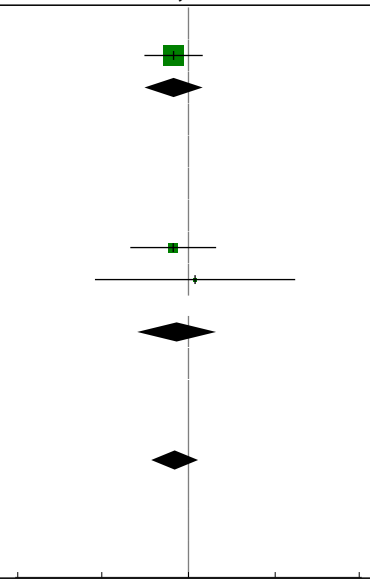
Analysis 3.3. Comparison 3 Biochemical response, Outcome 3 Alanine aminotransferase activity (Vitamin E vs other antioxidants) (IU/L).

Study or subgroup	Antioxidants N	Antioxidants Mean(SD)	Control N	Control Mean(SD)	Mean Difference Fixed, 95% CI	Weight	Mean Difference Fixed, 95% CI
3.3.1 Vitamin E trials							
Bugianesi 2005	28	-13 (8)	55	-50 (10)		65.81%	37[33.03,40.97]
Harrison 2003	23	-10 (60)	22	-30 (55)		0.92%	20[-13.61,53.61]
Vajro 2004	14	-24.1 (20.7)	14	-29.9 (31.7)		2.64%	5.83[-14,25.66]
Subtotal ***	**65**		**91**			**69.36%**	**35.59[31.72,39.46]**
Heterogeneity: Tau²=0; Chi²=9.96, df=2(P=0.01); I²=79.92%							
Test for overall effect: Z=18.04(P<0.0001)							
3.3.2 Other antioxidants trials							
Miglio 2000	96	-4.7 (19.3)	95	23.6 (23.4)		28%	-28.3[-34.39,-22.21]
Pamuk 2003	18	-18.2 (24.5)	17	-15 (40)		2.12%	-3.2[-25.33,18.93]
Rui 2001	22	-3.3 (90.1)	25	-64.4 (62.3)		0.52%	61.1[16.22,105.98]
Subtotal ***	**136**		**137**			**30.64%**	**-25.06[-30.88,-19.24]**
Heterogeneity: Tau²=0; Chi²=19, df=2(P<0.0001); I²=89.47%							
Test for overall effect: Z=8.44(P<0.0001)							
Total ***	**201**		**228**			**100%**	**17.01[13.79,20.23]**
Heterogeneity: Tau²=0; Chi²=318.41, df=5(P<0.0001); I²=98.43%							
Test for overall effect: Z=10.35(P<0.0001)							
Test for subgroup differences: Chi²=289.46, df=1 (P<0.0001), I²=99.65%							



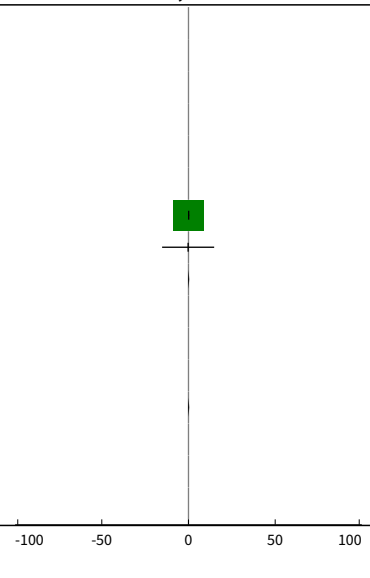
Analysis 3.4. Comparison 3 Biochemical response, Outcome 4 Gamma-glutamyl-transpeptidase activity (IU/L).

Study or subgroup	Antioxidants N	Antioxidants Mean(SD)	Control N	Control Mean(SD)	Mean Difference Fixed, 95% CI	Weight	Mean Difference Fixed, 95% CI
3.4.1 High quality							
Miglio 2000	96	-9.4 (54.4)	95	-0.8 (63.7)		64.68%	-8.6[-25.41,8.21]
Subtotal ***	**96**		**95**			**64.68%**	**-8.6[-25.41,8.21]**
Heterogeneity: Not applicable							
Test for overall effect: Z=1(P=0.32)							
3.4.2 Low quality							
Pamuk 2003	18	-16.4 (33)	17	-7.6 (41)		29.84%	-8.8[-33.54,15.94]
Rui 2001	22	-48.8 (108.2)	25	-52.6 (91.6)		5.48%	3.8[-53.94,61.54]
Subtotal ***	**40**		**42**			**35.32%**	**-6.84[-29.59,15.9]**
Heterogeneity: Tau²=0; Chi²=0.15, df=1(P=0.69); I²=0%							
Test for overall effect: Z=0.59(P=0.56)							
Total ***	**136**		**137**			**100%**	**-7.98[-21.5,5.54]**
Heterogeneity: Tau²=0; Chi²=0.17, df=2(P=0.92); I²=0%							
Test for overall effect: Z=1.16(P=0.25)							
Test for subgroup differences: Chi²=0.01, df=1 (P=0.9), I²=0%							



Analysis 3.5. Comparison 3 Biochemical response, Outcome 5 Serum total bilirubin levels (umol/L).

Study or subgroup	Antioxidants N	Antioxidants Mean(SD)	Control N	Control Mean(SD)	Mean Difference Fixed, 95% CI	Weight	Mean Difference Fixed, 95% CI
3.5.1 High quality							
Subtotal ***	**0**		**0**				**Not estimable**
Heterogeneity: Not applicable							
Test for overall effect: Not applicable							
3.5.2 Low quality							
Pamuk 2003	18	0.2 (0.6)	17	0.1 (0.4)		99.96%	0.1[-0.22,0.42]
Rui 2001	22	-14.2 (27.2)	25	-14 (25)		0.04%	-0.2[-15.21,14.81]
Subtotal ***	**40**		**42**			**100%**	**0.1[-0.22,0.42]**
Heterogeneity: Tau²=0; Chi²=0, df=1(P=0.97); I²=0%							
Test for overall effect: Z=0.62(P=0.54)							
Total ***	**40**		**42**			**100%**	**0.1[-0.22,0.42]**
Heterogeneity: Tau²=0; Chi²=0, df=1(P=0.97); I²=0%							
Test for overall effect: Z=0.62(P=0.54)							
Test for subgroup differences: Not applicable							

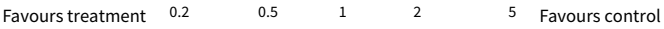


Comparison 4. Histological response

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Fibrosis persistence	1	45	Odds Ratio (M-H, Fixed, 95% CI)	0.76 [0.23, 2.46]
2 Fibrosis improvement	1	45	Mean Difference (IV, Fixed, 95% CI)	-0.25 [-0.91, 0.41]
3 Necro-inflammatory improvement	1	45	Mean Difference (IV, Fixed, 95% CI)	0.20 [-0.22, 0.62]

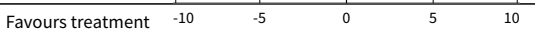
Analysis 4.1. Comparison 4 Histological response, Outcome 1 Fibrosis persistence.

Study or subgroup	Antioxidants n/N	Control n/N	Odds Ratio M-H, Fixed, 95% CI	Weight	Odds Ratio M-H, Fixed, 95% CI
Harrison 2003	12/23	13/22		100%	0.76[0.23,2.46]
Total (95% CI)	**23**	**22**		**100%**	**0.76[0.23,2.46]**
Total events: 12 (Antioxidants), 13 (Control)					
Heterogeneity: Not applicable					
Test for overall effect: Z=0.47(P=0.64)					



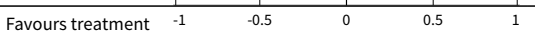
Analysis 4.2. Comparison 4 Histological response, Outcome 2 Fibrosis improvement.

Study or subgroup	Antioxidants N	Antioxidants Mean(SD)	Control N	Control Mean(SD)	Mean Difference Fixed, 95% CI	Weight	Mean Difference Fixed, 95% CI
Harrison 2003	23	-0.5 (1)	22	-0.2 (1.3)		100%	-0.25[-0.91,0.41]
Total ***	**23**		**22**			**100%**	**-0.25[-0.91,0.41]**
Heterogeneity: Not applicable							
Test for overall effect: Z=0.74(P=0.46)							



Analysis 4.3. Comparison 4 Histological response, Outcome 3 Necro-inflammatory improvement.

Study or subgroup	Antioxidants N	Antioxidants Mean(SD)	Control N	Control Mean(SD)	Mean Difference Fixed, 95% CI	Weight	Mean Difference Fixed, 95% CI
Harrison 2003	23	0 (0.7)	22	-0.2 (0.8)		100%	0.2[-0.22,0.62]
Total ***	**23**		**22**			**100%**	**0.2[-0.22,0.62]**
Heterogeneity: Tau^2=0; Chi^2=0, df=0(P<0.0001); I^2=100%							
Test for overall effect: Z=0.92(P=0.36)							

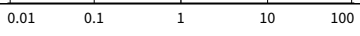


Comparison 5. Adverse events

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Headache	1	191	Odds Ratio (M-H, Fixed, 95% CI)	2.0 [0.18, 22.43]
2 Nausea	1	191	Odds Ratio (M-H, Fixed, 95% CI)	2.0 [0.18, 22.43]
3 Diarrhea	1	191	Odds Ratio (M-H, Fixed, 95% CI)	2.0 [0.18, 22.43]
4 Heartburn	1	191	Odds Ratio (M-H, Fixed, 95% CI)	0.99 [0.14, 7.17]
5 Meteorism	1	191	Odds Ratio (M-H, Fixed, 95% CI)	2.0 [0.18, 22.43]
6 Increase of transaminase	1	28	Odds Ratio (M-H, Fixed, 95% CI)	3.22 [0.12, 86.09]

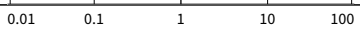
Analysis 5.1. Comparison 5 Adverse events, Outcome 1 Headache.

Study or subgroup	Antioxidants n/N	Control n/N	Odds Ratio M-H, Fixed, 95% CI	Weight	Odds Ratio M-H, Fixed, 95% CI
Miglio 2000	2/96	1/95		100%	2[0.18,22.43]
Total (95% CI)	**96**	**95**		**100%**	**2[0.18,22.43]**
Total events: 2 (Antioxidants), 1 (Control)					
Heterogeneity: Not applicable					
Test for overall effect: Z=0.56(P=0.57)					



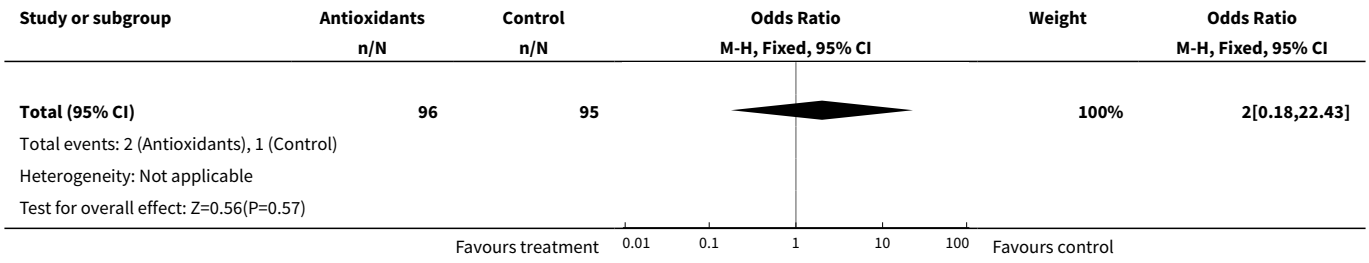
Analysis 5.2. Comparison 5 Adverse events, Outcome 2 Nausea.

Study or subgroup	Antioxidants n/N	Control n/N	Odds Ratio M-H, Fixed, 95% CI	Weight	Odds Ratio M-H, Fixed, 95% CI
Miglio 2000	2/96	1/95		100%	2[0.18,22.43]
Total (95% CI)	**96**	**95**		**100%**	**2[0.18,22.43]**
Total events: 2 (Antioxidants), 1 (Control)					
Heterogeneity: Not applicable					
Test for overall effect: Z=0.56(P=0.57)					

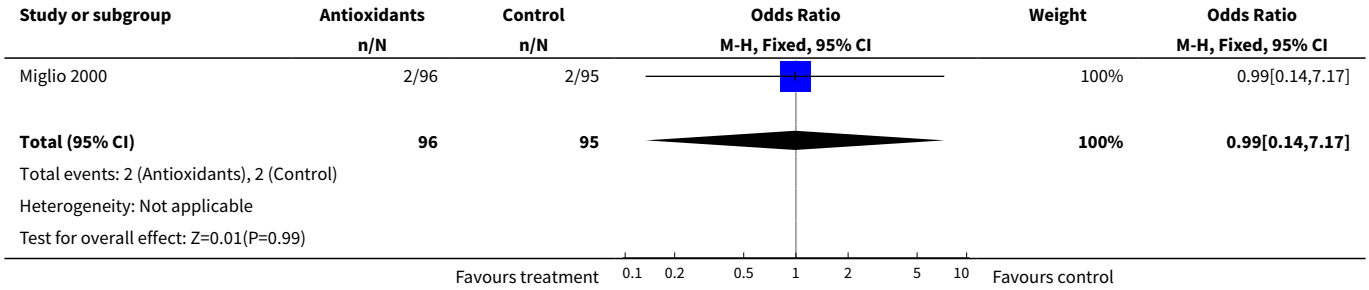


Analysis 5.3. Comparison 5 Adverse events, Outcome 3 Diarrhea.

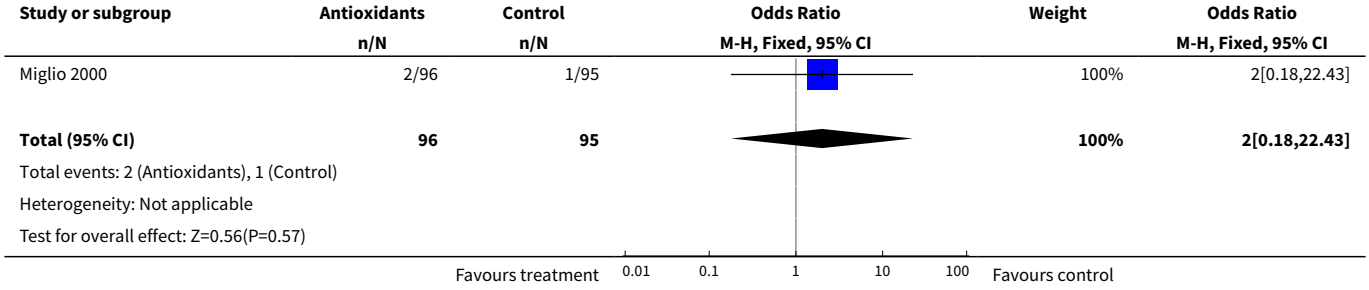
Study or subgroup	Antioxidants n/N	Control n/N	Odds Ratio M-H, Fixed, 95% CI	Weight	Odds Ratio M-H, Fixed, 95% CI
Miglio 2000	2/96	1/95		100%	2[0.18,22.43]



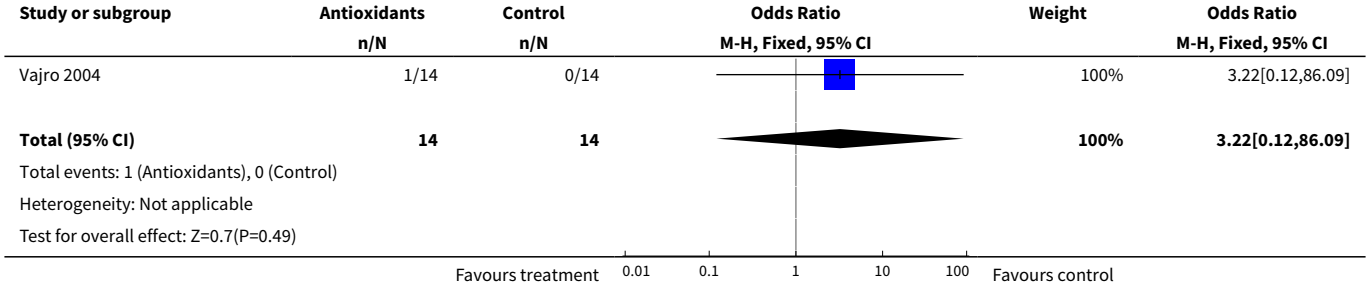
Analysis 5.4. Comparison 5 Adverse events, Outcome 4 Heartburn.



Analysis 5.5. Comparison 5 Adverse events, Outcome 5 Meteorism.



Analysis 5.6. Comparison 5 Adverse events, Outcome 6 Increase of transaminase.



APPENDICES

Appendix 1. Search strategies

Database	Time span	Search strategy
The Cochrane Hepato-Biliary Group Controlled Trials Register	June 2006.	(antioxidant* OR vitamin* OR N-acetylcysteine OR betaine OR glutathione OR S-adenosyl-methionine OR SAMe OR caroten* OR retinol OR silymarin OR selenium OR lycopene OR 'ascorbic acid' OR tocopherol OR flavonoid* OR polyphenol* OR ginseng) AND (('non*alcoholic fatty liver' OR NAFL OR NASH) OR steato*hepatitis)
Cochrane Central Register of Controlled Trials (CENTRAL) in The Cochrane Library	Issue 2, 2006.	#1 ANTIOXIDANTS explode all trees (MeSH) #2 VITAMINS explode all trees (MeSH) #3 BETAINE explode all trees (MeSH) #4 GLUTATHIONE explode all trees (MeSH) #5 SILYMARIN explode all trees (MeSH) #6 SELENIUM explode all trees (MeSH) #7 ASCORBIC ACID explode all trees (MeSH) #8 GINSENG explode all trees (MeSH) #9 (antioxidant* or vitamin* or n-acetylcysteine or betaine or glutathione or s-adenosyl-methionine or same or caroten* or retinol or silymarin or selenium or lycopene or (ascorbic next acid) or tocopherol or flavonoid* or polyphenol* or ginseng) #10 (#1 or #2 or #3 or #4 or #5 or #6 or #7 or #8 or #9) #11 ((non next alcoholic next fatty next liver) or nafl or nash) #12 steatohepatitis #13 (#10 and (#11 or #12))
MEDLINE	1966 to June 2006.	(antioxidant* OR vitamin* OR vitamin A OR "vitamin E" OR N-acetylcysteine OR betaine OR glutathione OR S-adenosyl-methionine OR SAMe OR carotene OR carotenoid* OR retinol OR silymarin OR selenium OR lycopene OR ascorbic acid OR tocopherol OR flavonoid* OR polyphenol* OR ginseng OR thioredoxin OR glutaredoxin OR 'black tea') AND ("non*alcoholic fatty liver" OR "non alcoholic fatty liver" OR "nonalcoholic fatty liver" OR "NAFL*" OR "NASH" OR (non*alcoholic steato*hepatitis OR (non alcoholic steato*hepatitis) OR (nonalcoholic steato*hepatitis) OR "bright liver" OR "liver disease")) AND (trial* OR stud* OR random* OR allocat* OR double*blind* OR placebo) NOT "NASH A" NOT "NASH AA" NOT "NASH AD" NOT "NASH AS" NOT "NASH B" NOT "NASH BR" NOT "NASH BW" NOT "NASH C" NOT "NASH CB" NOT "NASH CH" NOT "NASH CL" NOT "NASH CR" NOT "NASH CW" NOT "NASH D" NOT "NASH DC" NOT "NASH DF" NOT "NASH DL" NOT "NASH DR" NOT "NASH DT" NOT "NASH E" NOT "NASH EC" NOT "NASH EJ" NOT "NASH ES" NOT "NASH FD" NOT "NASH FW" NOT "NASH G" NOT "NASH GB" NOT "NASH General Hospital" NOT "NASH GL" NOT "NASH GS" NOT "NASH GV" NOT "NASH H" NOT "NASH HA" NOT "NASH Hall" NOT "NASH HD" NOT "NASH HM" NOT "NASH HW" NOT "NASH J" NOT "NASH JB" NOT "NASH JE" NOT "NASH JF" NOT "NASH JH" NOT "NASH JJ" NOT "NASH JM" NOT "NASH JQ" NOT "NASH JR" NOT "NASH JW" NOT "NASH K" NOT "NASH KA" NOT "NASH KL" NOT "NASH L" NOT "NASH LD" NOT "NASH LJ" NOT "NASH M" NOT "NASH MA" NOT "NASH MC" NOT "NASH ML" NOT "NASH MM" NOT "NASH MW" NOT "NASH N" NOT "NASH P" NOT "NASH PB" NOT "NASH PJ" NOT "NASH PP" NOT "NASH R" NOT "NASH RA" NOT "NASH reaction" NOT "NASH reagent" NOT "NASH RJ" NOT "NASH RS" NOT "NASH RW" NOT "NASH S" NOT "NASH SA" NOT "NASH SM" NOT "NASH solution" NOT "NASH SV" NOT "NASH T" NOT "NASH TA" NOT "NASH TC" NOT "NASH TD" NOT "NASH TE" NOT "NASH TG" NOT "NASH TL" NOT "NASH TW" NOT "NASH University" NOT "NASH W" NOT "NASH WA" NOT "NASH WG"

(Continued)

EMBASE	1980 to June 2006.	(antioxidant OR vitamin OR 'vitamin A' OR 'vitamin E' OR N-acetylcysteine OR betaine OR glutathione OR S-adenosyl-methionine OR SAMe OR carotene OR carotenoid OR retinol OR silymarin OR selenium OR lycopene OR 'ascorbic acid' OR tocopherol OR flavonoid OR polyphenol OR ginseng OR thioredoxin OR glutaredoxin OR 'black tea') AND ("non*alcoholic fatty liver" OR "non alcoholic fatty liver" OR "nonalcoholic fatty liver" OR "NAFL*" OR "NASH" OR (non*alcoholic steato*hepatitis) OR (non alcoholic steato*hepatitis) OR (non-alcoholic steato*hepatitis) OR (bright liver) OR liver disease)) AND (trial* OR stud* OR random* OR allocat* OR double*blind* OR placebo)
The Chinese Biomedical Database	1978 to June 2006.	Search strategy in Chinese. Available at a request from the Chinese Cochrane Center.

WHAT'S NEW

Date	Event	Description
13 August 2008	Amended	Converted to new review format.

CONTRIBUTIONS OF AUTHORS

F Lirussi designed the question, wrote the protocol, and supervised the writing of the review text. L Azzalini performed data extraction and statistical analysis, and co-wrote the review text. S Orando devised the search strategies and selected the trials. R Orlando was responsible for data retrieval. F Angelico provided statistical advice. All authors contributed to the improvement of the review.

DECLARATIONS OF INTEREST

None known.

INDEX TERMS

Medical Subject Headings (MeSH)

*Dietary Supplements; Antioxidants [*therapeutic use]; Fatty Liver [blood] [*drug therapy]; Randomized Controlled Trials as Topic

MeSH check words

Humans