

Signal Detection Report: Antioxidant supplements for non-alcoholic fatty liver disease and/or steatohepatitis

Signal Detection Report date

July, 2017

Key Findings

- Expert opinion: One expert stated the original review's recommendations were no longer valid, while another expert believed the findings were still valid but was aware of new evidence. A third expert did not know whether the original review's recommendations were still valid.
- Literature evidence: No signal was identified

Summary Decision: The review does not require updating at this time

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Introduction

Previous research evaluating a cohort of published systematic reviews demonstrated that 7% of reviews were out of date by the time of publication, while as many as 23% went out of date within two years of being completed.[1] The utility of systematic review-based evidence depends on their remaining up-to-date. As such, the Cochrane Complementary Medicine Field and the Knowledge Synthesis group at the Ottawa Hospital Research Institute (OHRI) have determined a set of existing reviews of interest for which signal detection work using the Ottawa Method's qualitative/quantitative signal detection approach has been performed. [1]

The Ottawa method involves identification of qualitative and quantitative signals/triggers indicating the need of updating of a systematic review.[1] The Ottawa method has been used to assess the need for updating systematic reviews in the past.[1; 2] A graphical overview of our approach and application of the Ottawa signal detection method is provided in Appendix A, Figure 1 of this report. This work assessed potential triggers signifying the need for updating of six past Cochrane Complementary Medicine reviews. The conclusion as to whether or not each of the reviews is in need of updating was made based on the identification of qualitative and quantitative signals.

Methods

We contacted content experts to provide input on whether conclusions from the review were still valid, and if they were aware of any new evidence that could potentially signal the need for a review update. This process included the authors from the original review, and also reached out to the lead authors on the included studies of the review. If we did not find sufficient number of experts we then reached out to those who had responded and asked whether they could suggest possible content experts.

Summaries and conclusions were collected for each key question (or possibly key endpoint) within each review; this summary was shared with consulting experts when seeking their input regarding the findings, as well as when establishing the presence of updating triggers. See Appendix B for the survey sent to content experts.

Experts were asked their perspectives on the following features of each review: a) whether the conclusion is still valid in their own opinion (to be answered as yes/no/don't know); b) whether he/she is aware of new evidence published since the publication date of the review being assessed (with details if yes); and c) any additional information or perspectives to be shared. If an expert indicated an opinion that one or more conclusions of the review was out of date and/or provided specific evidence they felt was critical to the decision regarding updating, we verified it by assessing the evidence brought forward by the expert(s).

Experts were given 10 business days to respond. Two reminders were sent, and a conflict of interest disclosure statement was also sent to all experts that participated. The survey was kept open until a minimum of two clinical experts responded.

One reviewer screened the evidence provided by the content experts, and determined whether the studies met the inclusion criteria of the original review. Data was extracted by one reviewer. The identification of qualitative signals was carried out using the Ottawa method qualitative signals. The definition and categories of qualitative signals are presented in Appendix C.

An information specialist provided modified PRESS [11] evaluations of the original review's search strategy, commenting on databases, search dates and terminology. This information is provided in Appendix D in order to inform any future updating for the original review.

Expert Opinion

Of the 11 experts contacted, three responded to our questions on antioxidant supplements for non-alcoholic fatty liver disease (NAFLD) and/or steatohepatitis (NASH). One expert felt the original review's conclusions were no longer valid. Another expert believed the findings were still valid, but wrote that he thought "more data [is] actually available confirming the positive effect of Vitamin E and other antioxidant agents (such as alpha lipoic acid and silymarin) not only on AST, but also on ALT levels." The expert went on to indicate that evidence has shown significant improvement in histological scores with Vitamin E and alpha lipoic acid. The final expert answered "don't know," and commented that "there are a lot of new trials published...but they are still very heterogeneous. Data must be meta-analyzed...to reject/confirm review results."

Flow

Eight references were put forth by the content experts. One publication did not meet the original review's inclusion criteria, and seven studies were assessed for qualitative signals.

Signal Detection Results:

Findings of the original review

Overall Conclusion of the Review: "There is insufficient data to either support or refute the use of antioxidant supplements for patients with NAFLD or NASH. Vitamin E may increase the activity of alanine aminotransferase in these patients. It may be advisable to carry out large prospective randomised clinical trials on this topic." [3]

Further Details: "Six randomised clinical trials were identified assessing the effects of antioxidant supplements for patients with NAFLD and NASH. There was 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. Moreover, the included trials varied substantially regarding methodological quality and hence bias risk - 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. Gammaglutamyl-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.”[3]

New findings:

Chachay et al., 2014 described a randomized, double-blind, placebo-controlled trial of overweight or obese men diagnosed with NAFLD. Twenty participants were included and randomized to receive 3000 mg resveratrol (n=10) or placebo (n=10) daily for 8 weeks. The authors reported that “eight-week administration of resveratrol did not reduce insulin resistance, steatosis, or abdominal fat distribution when compared with baseline. No change was observed in plasma lipids or antioxidant activity. Levels of alanine and aspartate aminotransferases increased significantly among patients in the resveratrol group until week 6 when compared with the placebo group. Resveratrol did not significantly alter transcription of NQO1, PTP1B, IL6, or HO1 in peripheral blood mononuclear cells. Resveratrol was well-tolerated.” The authors concluded that “eight weeks administration of resveratrol did not significantly improve any features of NAFLD, compared with placebo, but it increased hepatic stress, based on observed increases in levels of liver enzymes. Further studies are needed to determine whether agents that are purported to mimic calorie restriction, such as resveratrol, are safe and effective for complications of obesity.”[4]

Lavine et al., 2011 described a randomized, double-blind, double-dummy, placebo-controlled trial of patients aged 8-17 years with diagnosed NAFLD. 173 participants were randomized to receive 400 IU of vitamin E and placebo (n = 58), 500 mg of metformin and placebo (n = 57), or both placebos (n = 58) twice daily for 96 weeks of treatment. Participants were followed for another 24 weeks after treatment ceased. The authors report that “sustained reduction in ALT level was similar to placebo (10/58; 17%; 95% CI 9% to 29%) in both the vitamin E (15/58; 26%; 95% CI 15% to 39%; $P=0.26$) and metformin treatment groups (9/57; 16%; 95% CI 7% to 28%; $P=0.83$). The mean change in ALT level from baseline to 96 weeks was -35.2 U/L (95% CI -56.9 to -13.5) with placebo vs -48.3 U/L (95% CI -66.8 to -29.8) with vitamin E ($P=0.07$) and -41.7 U/L (95% CI -62.9 to -20.5) with metformin ($P=0.40$). The mean change at 96 weeks in hepatocellular ballooning scores was 0.1 with placebo (95% CI -0.2 to 0.3) vs -0.5 with vitamin E (95% CI -0.8 to -0.3 ; $P=0.006$) and -0.3 with metformin (95% CI -0.6 to -0.0 ; $P=0.04$); and in NAFLD activity score, -0.7 with placebo (95% CI -1.3 to -0.2) vs -1.8 with vitamin E (95% CI -2.4 to -1.2 ; $P=0.02$) and -1.1 with metformin (95% CI -1.7 to -0.5 ; $P=0.25$). Among children with NASH, the proportion who resolved at 96 weeks was 28% with placebo (95% CI 15% to 45%; 11/39) vs 58% with vitamin E (95% CI 42% to 73%; 25/43; $P=0.006$) and 41% with metformin (95% CI 26% to 58%; 16/39; $P=0.23$). Compared with placebo, neither therapy demonstrated significant improvements in other histological features.” The authors concluded that “neither vitamin E nor metformin was superior to placebo in attaining the primary outcome of sustained reduction in ALT level in patients with pediatric NAFLD.”[5]

Sanyal et al., 2010 reported a pivotal randomized controlled trial of adult patients without diabetes who had been diagnosed with NASH. 247 subjects were randomized to receive 30 mg of pioglitazone and placebo (n = 80), 800 IU of vitamin E and placebo (n = 84), or double-placebo (n = 83) once daily for 96

weeks. Subjects were followed for 24 weeks after treatment ceased. Authors report that “vitamin E therapy, as compared with placebo, was associated with a significantly higher rate of improvement in nonalcoholic steatohepatitis (43% vs. 19%, $P = 0.001$), but the difference in the rate of improvement with pioglitazone as compared with placebo was not significant (34% and 19%, respectively; $P = 0.04$). Serum alanine and aspartate aminotransferase levels were reduced with vitamin E and with pioglitazone, as compared with placebo ($P < 0.001$ for both comparisons), and both agents were associated with reductions in hepatic steatosis ($P = 0.005$ for vitamin E and $P < 0.001$ for pioglitazone) and lobular inflammation ($P = 0.02$ for vitamin E and $P = 0.004$ for pioglitazone) but not with improvement in fibrosis scores ($P = 0.24$ for vitamin E and $P = 0.12$ for pioglitazone). Subjects who received pioglitazone gained more weight than did those who received vitamin E or placebo; the rates of other side effects were similar among the three groups. The authors concluded that “Vitamin E was superior to placebo for the treatment of nonalcoholic steatohepatitis in adults without diabetes. There was no benefit of pioglitazone over placebo for the primary outcome; however, significant benefits of pioglitazone were observed for some of the secondary outcomes.”[6]

Hoofnagle et al., 2013 reported a post hoc analysis of Sanyal et al., 2010 [6], a double-blind, double-dummy randomized trial of nondiabetic adult patients diagnosed with NASH. This analysis focused on the patients who received vitamin E (800 IU) and pioglitazone placebo ($n=84$) compared to both placebos ($n=83$) once daily for 96 weeks. The authors reported that “ALT responses were more frequent among vitamin E (48%) than placebo (16%) recipients ($P < 0.001$). Among vitamin E recipients, ALT responses were associated with decreases in NAS ($P < 0.001$), but not fibrosis scores ($P = 0.34$), whereas among placebo recipients, ALT responses were associated with significant decreases in both ($P < 0.05$). Weight loss (≥ 2 kg) was also associated with ALT response ($P < 0.001$), improvements in NAS ($P < 0.001$) and fibrosis ($P < 0.02$), but vitamin E had an added effect both with and without weight loss. Weight gain (≥ 2 kg) was associated with lack of ALT response and worsening NAS and fibrosis scores in patients not on vitamin E.” The authors concluded that “Vitamin E can improve both ALT levels and histology with and without weight loss.”[7]

Solhi et al., 2014 described a randomized controlled trial of adult patients diagnosed with NASH. 64 participants were randomized to receive 70 mg of silymarin ($n = 33$) or placebo ($n = 31$) three times per day for 8 weeks. The authors reported that “serum concentrations of ALT were 91.3 ± 21.3 and 38.4 ± 11.8 in case group before and after the study respectively, while the figures were 84.6 ± 23.3 and 52.3 ± 29 in the control group ($P=0.026$). The same trend was seen for AST ($P=0.038$).” The authors concluded that “the patients who had taken silymarin experienced more notable fall in hepatic enzymes.”[8]

Hajiaghahmohammadi et al., 2012b reported a randomized controlled pilot study of adult patients diagnosed with NAFLD. 66 participants were randomized to receive 15 mg of pioglitazone ($n = 22$), 500 mg of metformin ($n = 22$), or 140 mg of silymarin ($n = 22$) once daily. The authors reported that “after the intervention, a significant reduction was observed in average amount of FBS, lipid profile, ALT, AST, serum insulin level and HOMA index in all three groups ($P < 0.01$). The most reduction in average FBS, TG, serum insulin level, and HOMA index was observed in pioglitazone group, the most reduction in average amount of cholesterol was seen in metformin group, and the most decrease in average amount

of AST and ALT occurred in silymarin group.” The authors concluded that “these results suggest that all drugs are beneficial in improving biochemical indices in patients with NAFLD. Changes in AST and ALT in silymarin group were demonstrated more than that in other groups and the average difference between changes was significant between silymarin and metformin groups.”[9]

Hajiaghamohammadi et al., 2012a described a double blind randomized trial of adult patients diagnosed with NAFLD. 66 patients were randomized to receive 2 g of licorice root extract (n = 33) or placebo (n = 33) once daily for two months. The authors reported that “in the case group, the mean alanine aminotransferase (ALT) level decreased from 64.09 to 51.27 IU/mL and the aspartate aminotransferase (AST) level decreased from 58.18 to 49.45 IU/mL, which were statistically significant ($p < 0.001$ and $p < 0.001$). But in the control group, a drop in the ALT and AST levels was not statistically significant. The BMI difference before and after the study was not statistically significant in both groups.” The authors concluded that “despite the significant drop in liver enzymes following administration of licorice root extract, it is recommended that further studies that include histological examination are necessary.”[10]

Adverse effects:

Resveratrol: No safety alerts were reported for Resveratrol. In Chachey et al.[4] mild gastrointestinal symptoms were reported by 80% of subjects in the resveratrol group compared with 20% in the placebo group.

Vitamin E: No safety alerts were reported for Vitamin E. Lavine et al.[5] compared development of diabetes, change in BMI, elevations in ALT level 2-fold or greater from baseline, or need for hospitalization or surgery and reported that “differences between treatment groups in terms of frequency or severity of adverse events were not significant.”[5] Sanyal et al.[6] described 19 severe adverse events, and none of these differed significantly between the treatment groups. 8 patients withdrew from the study (none from the Vitamin E group) and one patient from the vitamin E group died due to sepsis.

Other antioxidants: There were no safety alerts identified for silymarin or licorice. Hajiaghamohammadi et al.[9] reported no side effects for silymarin treatment, and Hajiaghamohammadi et al.[10] did not describe adverse effects of licorice. However, the study did report that no patients in either group dropped out.

Quantitative Signal: No signal was identified

New evidence was mainly consistent with the original review findings. New data from Chachay et al., 2014 was added to the AST meta-analysis, and the new pooled estimate remained statistically significant (MD= -4.44, 95% CI -7.88, -1.01)(Figure 2). It is important to note that the population in this study was overweight or obese men, so applicability may be a concern. **No Signal [4]**

Given that it was unclear if the original meta-analysis referred to mean change at end of follow-up or mean change from baseline, we have illustrated a second meta-analysis (Figure 3) adding the calculated mean change from baseline for Chachay et al., 2014. The pooled estimate remained statistically significant in favour of resveratrol (MD = -3.56, 95% CI -6.88, -0.24).

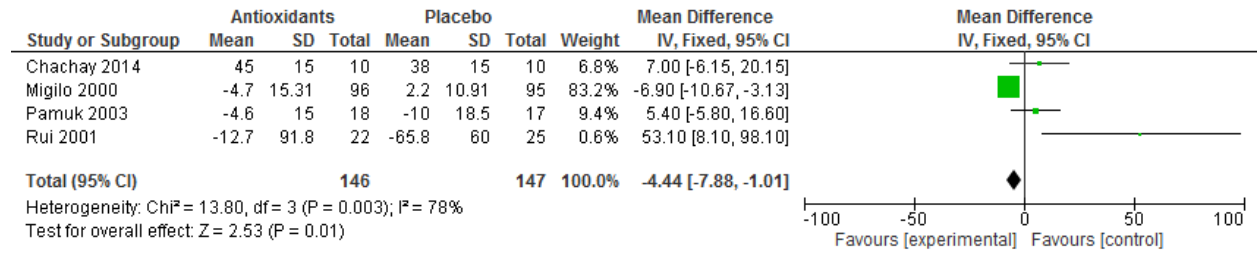


Figure 2: Aspartate aminotransferase (AST), mean change at end of follow-up

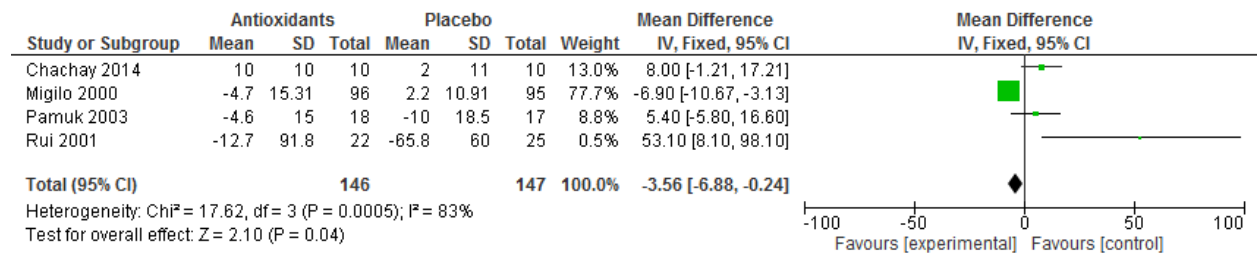


Figure 3: Aspartate aminotransferase (AST), mean change from baseline

Data for ALT was consistent with the original review’s overall findings of increase in ALT. We were unable to pool the data for ALT because it was reported in a different format (median and range instead of mean and SD). **No Signal [4]**

We could not assess the quantitative signal for ALT in three studies because the outcome data provided were incomplete (SD not provided and were unavailable from the authors), and thus these studies could not be added to the meta-analysis from the original review.[5,9,10]

Solhi et al., 2014 reported that “serum concentrations of ALT were 91.3±21.3 and 38.4±11.8 in the case group before and after the study, respectively, while corresponding values of 84.6±23.3 and 52.3±29 were observed in the control group (P=0.026). The same trend was seen for AST (P=0.038).”[8] When added to the meta-analysis from the original review, results did not change notably (MD = 12.37, 94% CI 9.35, 15.38, p < 0.00001) (Figure 4). The magnitude of the effect estimate changed from MD=17.01 to MD= 12.37, however the direction and statistical significance level remained the same. **No. Signal [8]**

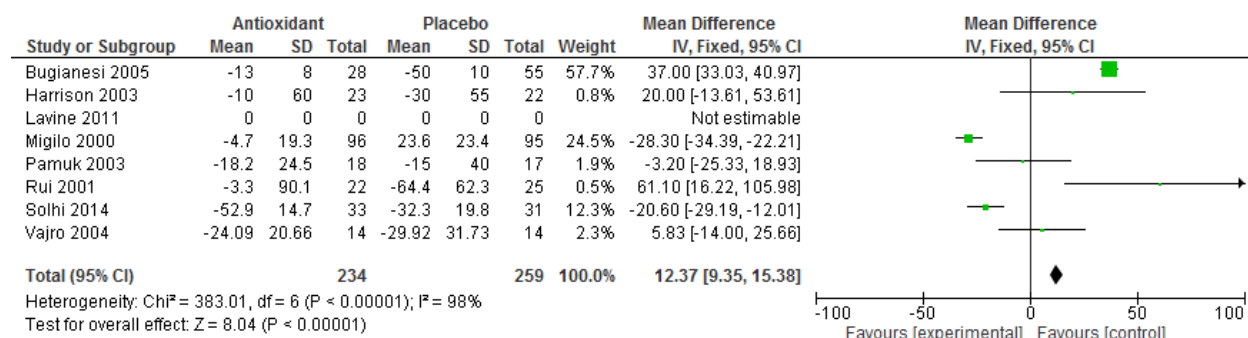


Figure 4: Alanine aminotransferase (ALT) activity

Qualitative Signal: No signal was identified

There was heterogeneity among the identified trials with respect to age (children and adults) and interventions (different types of antioxidants such as vitamin E, silymarin, and licorice root). Of the three studies assessing vitamin E, two analyzed data from the same trial and concluded that vitamin E was superior to placebo for the treatment of NASH[5, 6]. **Sanyal et al., 2010**, a pivotal trial, reported similar results to the original review in terms of no significant difference for improvement in fibrosis scores, and reduction of AST levels in Vitamin E arm (the data cannot be pooled.) Authors reported a significant reduction in lobular inflammation (an outcome that is not reported in the original review) for vitamin E versus placebo, but the data was too limited to generate any signal. Opposite to the original review’s finding of increase in ALT in the subgroup analysis for the Vitamin E arm, this study showed a reduction in ALT (data could not be pooled) [6]. Hoofnagle et al., 2013 is a post hoc analysis of an RCT and did not report any direct data on outcomes of interest [7].

One study looked at the effect of vitamin E compared to metformin or placebo in children [7-17 years old] with NAFLD, and concluded that neither treatment was superior to placebo in attaining a reduction in ALT levels[5]. However, it reported a significantly higher proportion of children with NAFLD that were resolved in Vitamin E compared to placebo group [5]. This was not an outcome of interest in the original review.

The single trial[4] studying Resveratrol for overweight or obese men with NAFLD found that ALT and AST levels increased significantly to week 6 (56% and 50% median increase) in the resveratrol group. However, the statistical significance was not sustained for overall change across study after the 8 week follow-up. [4]Two studies assessed the antioxidant silymarin in comparison with placebo[8] or metformin and pioglitazone[9]. In the first study, ALT and AST levels before and after treatment with silymarin or placebo both fell significantly (p = 0.026 and p = 0.038)[8]. In the second study, AST and ALT levels fell in all three treatment groups, but the greatest change occurred in the silymarin group, with changes significantly larger relative to both other groups (p = 0.003 and p = 0.005)[9]. The findings from these two studies were consistent with the original review for AST and ALT (other antioxidants subgroup). Neither study assessed outcomes related to other biochemical response or histology.

Finally, one study[10] investigated the effects of licorice root compared to placebo for NAFLD. There was a significant drop in both ALT and AST levels in the licorice group (within group comparison only).

Histology was not studied, and authors recommended further research to determine whether licorice root could have an impact on liver function and histology.

Conclusion: No qualitative and quantitative signal was identified. As such, the conclusions of the original review remains unchanged and it does not require updating at this time.

References:

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Appendix A: Overview of the Modified Ottawa Method

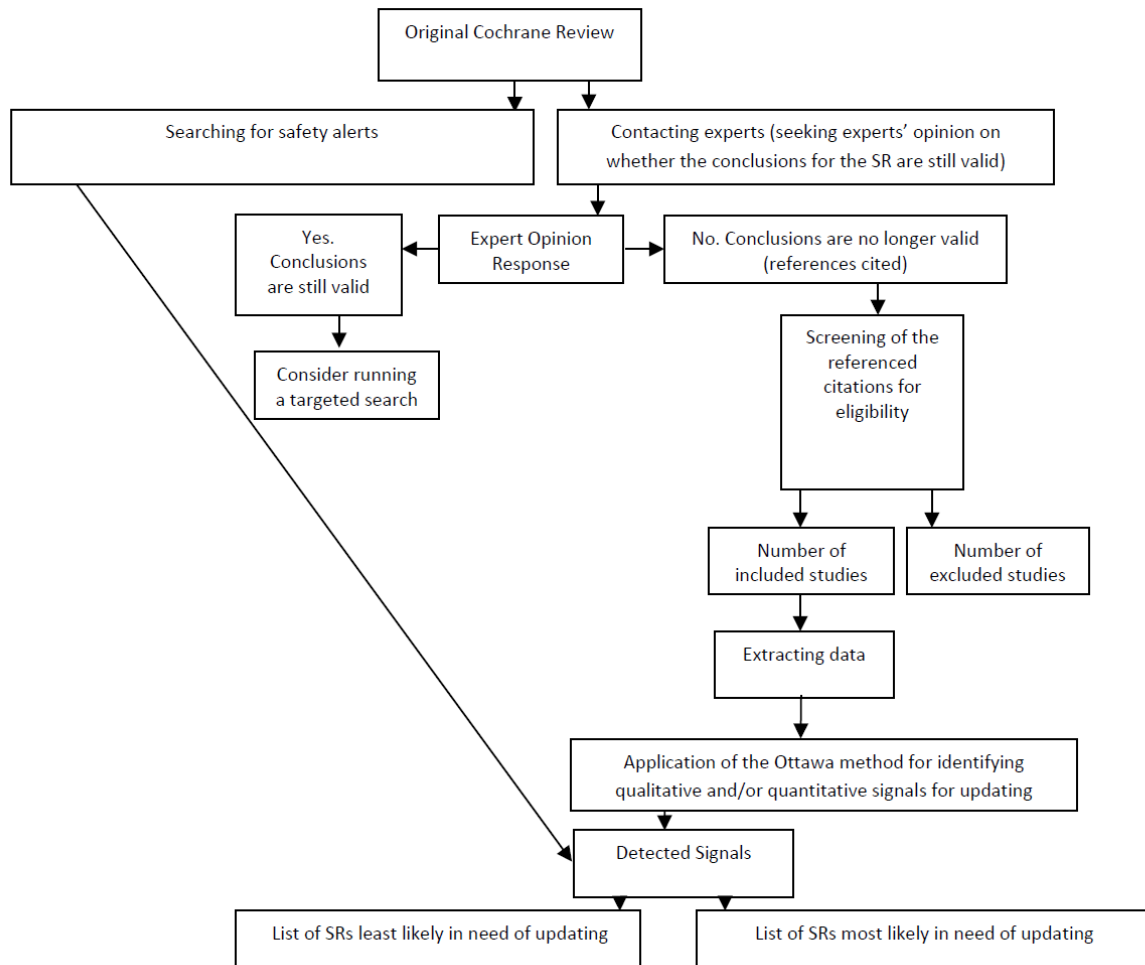


Figure 1: The process of signal detection methods for Cochrane reviews

Appendix B: Expert Opinion Survey

Conclusions from systematic review	Is the conclusion(s) in this review still valid? (Yes/No/Don't know)	Are you aware of any new evidence that is sufficient to invalidate the finding(s) in this review? (Yes/No/Don't know) If yes, please provide references	Comments
Review Objective: To systematically evaluate the beneficial and harmful effects of antioxidant supplements versus no intervention, placebo, or other interventions for patients of any age, sex, or ethnic origin NAFLD, including NASH and/or cryptogenic cirrhosis.			
<p>Overall Conclusion of the Review: There is insufficient data to either support or refute the use of antioxidant supplements for patients with NAFLD or NASH. Vitamin E may increase the activity of alanine aminotransferase in these patients. It may be advisable to carry out large prospective randomised clinical trials on this topic.</p> <p><u>Further Details:</u> Six randomised clinical trials were identified 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. Moreover, the included trials varied substantially regarding methodological quality and hence bias risk - 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. Gammaglutamyl-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</p>			

<p>of these agents. Adverse events were non-specific and of no major clinical relevance.</p>			
<p>All-cause mortality and liver-related mortality: None of the included trials reported any fatalities related or unrelated to liver disease.</p>			
<p>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).</p>			
<p>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</p>			

<p>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.</p>			
<p><u>Histological Response:</u> 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.</p>			
<p><u>Quality-of-life measures and cost effectiveness</u> Quality of life measures and cost effectiveness were not reported in any of the trials.</p>			
<p><u>Adverse Events:</u> Among the six trials, four provided information on adverse events. In the trials by Harrison et al (Harrison 2003) and</p>			

<p>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.</p>			
<p>Abbreviations: ALT (Alanine aminotransferase) AST (Aspartate aminotransferase); GGT (Gamma-glutamyl-transpeptidase); Non-alcoholic fatty liver disease (NAFLD); nonalcoholic steatohepatitis (NASH); SD (standard deviation); US (ultrasonography); WMD (weighted mean difference)</p>			

Appendix C: The Ottawa Method Qualitative Signals*

Potentially invalidating change in evidence

This refers to a situation in which it is expected that clinicians do not act upon the results of the original systematic review (SR) and the agency/organization that supported the original production of the SR would retract the SR until it is updated. Criteria for potentially invalidating change in evidence are presented below.

This category of signals (A1-A3) specifies findings from a pivotal trial**, meta-analysis (with at least one new trial), practice guideline (from major specialty organization or published in peer-reviewed journal), or recent textbook (e.g., *UpToDate*):

- Opposing findings (e.g., effective vs. ineffective) – **A1**
- Substantial harm (e.g., the risk of harm outweighs the benefits) – **A2**
- A superior new treatment (e.g., new treatment that is significantly superior to the one assessed in the original CER) – **A3**

Major change in evidence

This category of signals (A4-A7) refers to situations in which there is a clear potential for the new evidence to affect the clinical decision making. These signals, except for one (A7), specify findings from a pivotal trial, meta-analysis (with at least one new trial), practice guideline (from major specialty organization or published in peer-reviewed journal), or recent textbook (e.g., *UpToDate*):

- Important changes in effectiveness short of “opposing findings” – **A4**
- Clinically important expansion of treatment (e.g., to new subgroups of subjects) – **A5**
- Clinically important caveat – **A6**
- Opposing findings from meta-analysis (in relation to a meta-analysis in the original CER) or non-pivotal trial – **A7**

* Please, see Shojania et al. 2007 for further definitions and details

**A pivotal trial is defined as: 1) a trial published in top 5 general medical journals such as: Lancet, JAMA, Annals of Intern Med, BMJ, and NEJM. Or 2) a trial not published in the above top 5 journals but have a sample size of at least triple the size of the previous largest trial in the original CER.

Appendix D: Modified PRESS Review for Original Review's Search Strategy

The original search was executed in June 2006 in multiple databases. The time spans for each of the databases is listed but the precise date on which the search was performed is not. The full strategies are available in an appendix but are missing details as to the date run and the number of hits per line.

There are potential terms within the antioxidant category that have been missed and although a list is provided (p. 3, Types of Interventions), it does not appear definitive. NCCIH lists some examples <https://nccih.nih.gov/health/antioxidants/introduction.htm> (vitamins C and E, selenium, and carotenoids, such as beta-carotene, lycopene, lutein, and zeaxanthin) but not all of these terms appear in the search strategies. Of the antioxidants included, many seem to be missing synonyms: Betaine (e.g., acidin-pepsin, cystadane, lycine); glutathione (e.g., glutathine, glutathiol, tationil); silymarin (e.g., carsil, flavobion, legalon); selenium (e.g., 80 Se, radioselenium, selenicum); ascorbic acid (e.g., ascorbate sodium, hybrin, magnorbin); and ginseng (e.g., jen shen, ninjin, renshen). Ascorbic acid in particular has an extensive list of synonyms in Embase that have not been incorporated. An obvious alternate spelling, anti-oxidant*, has also been missed. Acetylcysteine seems a relevant MeSH, but neither it nor a full range of key words has been used.

There are inconsistencies across the search strategies (e.g., the Cochrane specialized register and CENTRAL searches do not include terms for "bright liver" "liver disease"). The MEDLINE and Embase strategies are more similar but do not include field names and presumably default to multi-field (mp) searching. This approach does not harness the power of using controlled vocabulary (e.g., exploding terms, utilizing subheadings), although the Cochrane strategy, which utilizes MeSH, partially mitigates this. The MEDLINE strategy is particularly unique through its exclusion of extensive list of NASH variants, presumably to remove unwanted results. However, there are dangers in "NOTing" out terms that might be shared within a record. Some of the excluded phrases, e.g., "Nash A", would pick up Nash at the end of the word followed by a sentence beginning with the word "A". Several potentially relevant records were lost this way and at least one other by noting out "Nash DR". "SAME" is used in all the searches but has the potential for generating many false hits without pairing it with other vocabulary to contextualize its meaning.

In addition, there are many opportunities for word variations and plurals missed (e.g., flavonoid, polyphenol, vitamin). There are also some redundancies, e.g., "vitamin" on its own as well as specific phrases such as "Vitamin A" and "Vitamin E" (but not "Vitamin C", which fits ascorbic acid). These redundancies did not have any effect on the search.

In conclusion, the strategy has some significant limitations with regard to vocabulary and it is possible that not all the relevant antioxidants were considered and incorporated. The documentation lacks transparency through its exclusion of information regarding the date run and the number of hits per line. These deficiencies increase the likelihood of missing potentially relevant records.