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Vadarlis A, Antza C, Bakaloudi DR, et al. Systematic review with meta-analysis: The effect of vitamin E supplementation in adult patients with non-alcoholic fatty liver disease. *Journal of Gastroenterology and Hepatology*. 2021;36(2):311-319.

What We Know, Think We Know, or Are Starting to Know

2022 has been slipping by and we have yet to top-up the fatty liver catalogue. Of course, we are interested in how to get fat *out* of the liver, and the nutritional strategies that may be useful to improving the overall spectrum that is fatty liver disease [1].

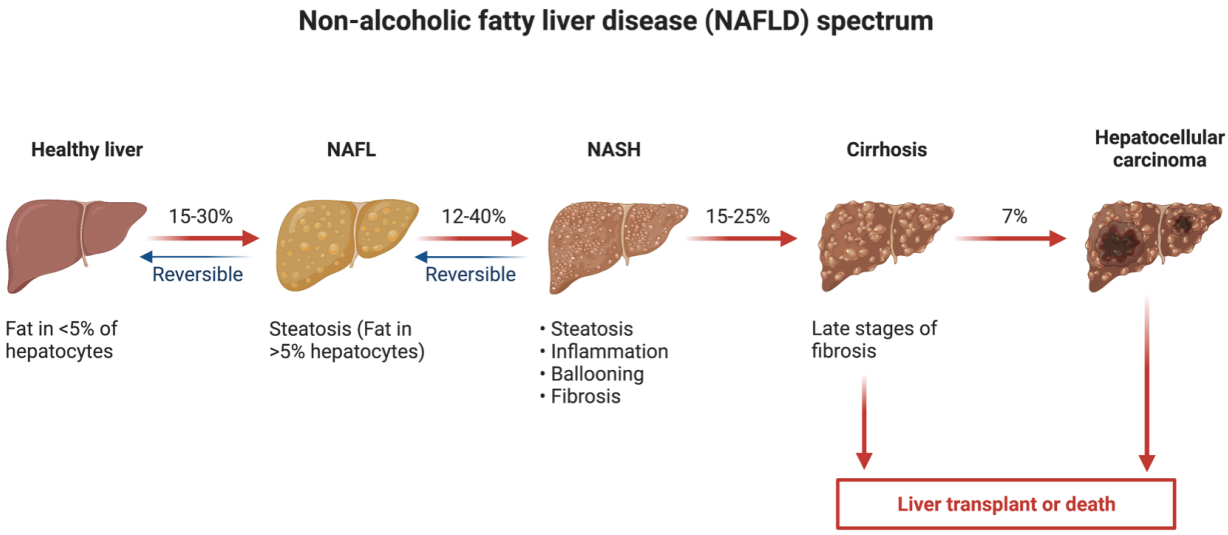


***Figure** illustrating the spectrum of fatty liver disease, from the initial progression to steatosis, i.e., non-alcoholic fatty liver [NAFL] which is diagnosed as the presence of intracellular fat in >5% of liver cells [hepatocytes], to liver cirrhosis and cancer. The percentages between each stage represent broad estimates of the incidence ranges in the general population. NAFL and NASH are the main focus from a nutritional perspective due to these stages being reversible. The difference between NAFL and NASH is that in addition to the steatosis, NASH is characterised by ballooning [an abnormal enlargement of liver cells, hepatic inflammation, and fibrosis [scarring of liver tissue].*

Much of the nutritional focus for the presence, and removal, of fat in the liver emphasises total energy intake and macronutrient composition. Indeed, the primary driver of increasing fat storage in the liver is excess energy, independent of the macronutrient source, as we covered in a previous Deepdive (see also this Deepdive on effects during energy balance, and this Deepdive on a meta-analysis of macronutrient effects on liver fat).

But what of micronutrients? In fact, there is a decent body of evidence for the use of supplemental vitamin E, particularly in patients with NASH [2,3]. As the major fat-soluble antioxidant, vitamin E exerts several effects which may improve the clinical presentations of NAFLD/NASH, including anti-oxidative and anti-inflammatory activities [2,3]. The present study we look at is the most recent meta-analysis of vitamin E supplementation in NAFLD/NASH.

*Geek Box: Pathways of Liver Fat Accumulation

In the fasted state, adipose tissue lipolysis [i.e., the breakdown of stored TGs and release of free fatty acids] constitutes the primary endogenous pathway delivering NEFA to the liver. As humans spend most of the day in the fed state, however, it is important to look at the various pathways through which fatty acids may be delivered to the liver from dietary intake.

There are three main pathways:

- *chylomicron-spillover NEFA*
- *chylomicron remnants*
- *de novo lipogenesis [DNL]*

The chylomicron pathways are derived from dietary fat. Dietary fat in the form of triglycerides [TGs] enters circulation from the intestines packaged into chylomicrons, large triglyceride-rich lipoproteins which constitute the pathway of dietary fat intake. TGs in chylomicrons are hydrolysed [i.e., broken down] into NEFA by a group of enzymes known as lipases, in particular lipoprotein lipase [LPL].

A proportion of fatty acids mobilised from LPL acting on chylomicron-TGs are not taken up by adipose tissue, and "spillover" into the pool of circulating NEFA which contributes the greatest proportion of fatty acids to intra-hepatic triglycerides [IHTG]. In general, the contribution of systemic NEFA to hepatic fatty acids may be in the region of 45-75%.

This pathway of LPL-mediated breakdown of chylomicron-TGs also produces what are known as "remnants", formed when the hydrolysis of chylomicron-TGs results in a smaller lipoprotein, i.e., a chylomicron-remnant. These chylomicron-remnants are taken up by the liver, and the remaining TGs in the remnant particle may be repackaged into VLDL. Over a 24-hr period, the contribution of NEFA derived from chylomicron-remnants has been shown to be greater than the contribution of chylomicron spillover NEFA.

The final exogenous pathway is DNL, where fatty acids are synthesised in the liver from non-fat precursor sources, primarily from excess dietary carbohydrate, in particular free sugars [proteins contribute very little to DNL]. The contribution of DNL to hepatic NEFA in metabolically healthy individuals is relatively small at <5%, however, the presence of fatty liver substantially modifies the rate of DNL, which may be up to ~22-24% in individuals with NAFLD. In addition, insulin resistance strongly modifies post-prandial DNL, which increases in individuals with elevated insulin levels.

In sum, hepatic fat originates from endogenous systemic NEFA derived from adipose tissue and splanchnic lipolysis, exogenous dietary fatty acids derived from chylomicron spillover or chylomicron remnants, and the de novo synthesis of fatty acid from non-fat precursors, in particular carbohydrate. The respective contribution of fatty acids to VLDL-TG have been shown to be in the region of 75-84% from the systemic NEFA pool, 12-39% from dietary fatty acids, and 5-22% from DNL, with the range of contributions reflecting variability due to metabolic health of the individual, in addition to dietary composition.

The Study

The investigators conducted a systematic review of vitamin E supplementation according to the following inclusion criteria:

- **Design**: randomised controlled trials
- **Population**: adults with a diagnosis of NAFL or NASH
- **Intervention**: vitamin E in either tocopherol and/or tocotrienol forms
- **Comparison**: a placebo control
- **Outcome**: the primary outcome was change in levels of the liver enzymes, alanine aminotransferase [ALT] and aspartate aminotransferase [AST]

Elevated ALT/AST reflect damage to the liver. Secondary outcomes included liver fat levels, fibrosis, inflammation, and ballooning. The investigators also conducted several subgroup analyses, including according to baseline ALT/AST levels and distinguishing between NASH and NAFL.

Results: Eight studies were included in the systematic review, of which seven studies were included in the meta-analysis. Of these, four trials were conducted in patients with NAFL, while three were in patients with NASH. The study durations ranged from 3-months to 2-years, and doses of vitamin E between 400-800IU/d used in five studies, while one used mixed tocotrienols and another used delta-tocotrienol.

- ***Effect on ALT/AST*:** Based on seven studies, vitamin E supplementation resulted in a significant decrease of ALT by 7.37IU/L [95% CI, 4.64 to 10.11], and significant decrease of AST by 5.71IU/L [95% CI, 1.93 to 9.49].

In subgroup analysis of 4 studies with participants with elevated ALT/AST above normal ranges at baseline, the effect was stronger: a 12.40IU/L [95% CI, 5.74 to 19.06IU/L] and 9.46IU/L [95% CI, 2.76 to 16.15IU/L] reduction in ALT/AST, respectively.

Confining the analysis just to participants with NASH [three trials], the effect was stronger again: a 16.49IU/L [95% CI, 6.27 to 26.71IU/L] and 12.78IU/L [95% CI, 4.93 to 20.62IU/L] for ALT/AST, respectively [more under ***Key Characteristic***, below].

- ***Effect on Liver Fat*:** Based on three studies, vitamin E supplementation lowered liver fat by 0.61% [95% CI. 0.34% to 0.89%].
- ***Effect on LDL-C*:** Based on five studies, vitamin E supplementation lowered LDL-C by 4.39mg/dL [95% CI, 2.1 to 6.67mg/dL].

Vitamin E supplementation also resulted in significantly lower hepatic inflammation and liver ballooning, and significant reductions in ALT/AST were observed for both tocopherol and tocotrienol vitamin E isoforms [more under ***Interesting Finding***, below].

The Critical Breakdown

Pros: The study provides the most updated synthesis of the evidence to date. The inclusion criteria was clearly defined, and the literature search was conducted using relevant research databases. The methodological quality and risk of bias was systematically assessed for each included study. Effect sizes were reported with confidence intervals. A sensitivity analysis was conducted excluding one study at high risk of bias, and this did not alter the primary outcome. The subgroup analyses allowed for further insights based on baseline ALT/AST levels, and whether patients had NASH specifically.

Cons: There is some shoddy reporting in the paper. They state the subgroup analysis was predefined, but do not state what exactly was predefined. There was no mention of the sex of participants, which is relevant because the reference ranges for ALT/AST differ based on sex. Aside from two studies that specified the isoform of vitamin E, the other studies were defined only as "vitamin E" without stating whether this was isolated alpha-tocopherol or mixed isoforms. The numbers of included participants also don't quite add up; they say the eight studies totalled 465 participants, but this is way off the data presented in Table 1, and then say the total number of participants in the seven studies meta-analysed for the primary outcome was 465. This could just be typos and shoddy reviewing, but these are important details. The study also did not clarify what fibrosis score was used, which differs relative to the severity of underlying liver disease. In fact, they don't state what the unit of measurement for multiple secondary outcomes are, which is unfathomable how that got passed peer review. Some of the included studies also had energy restriction as part of the dietary recommendations, thus it is difficult to attribute effects such as liver fat reduction to vitamin E alone. The overall sample size of the included studies is small.

Key Characteristic

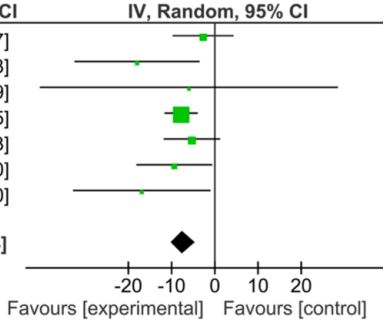
The subgroup analysis was a useful distinction to make, given that the weight of evidence for vitamin E supplementation and current practice guidelines to date are confined to biopsy-proven NASH [4]. Nevertheless, recall from above that fatty liver disease is a spectrum, and the increasing fat in the liver which characterises steatosis *per se* is also accompanied with increased oxidative stress and inflammation, which may damage the liver over time in the progression to NASH [1–3].

Thus, the fact that the primary outcome of lowering ALT/AST was observed in the present meta-analysis, in participants with steatosis [fatty liver, NAFL] rather than NASH, suggests vitamin E may provide modest but clinically important reductions in ALT/AST. In fact, the study above which showed the least effect – Anushiravan *et al*. 2019 – had participants with the lowest baseline levels.

a

Study or Subgroup	Vitamin E Mean	SD	Total	Placebo Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
Anushiravani 2019	-3.3	13.3	30	-0.6	14.6	30	15.0%	-2.70 [-9.77, 4.37]
Bril 2019	-25	31.04	36	-7	29.8	32	3.6%	-18.00 [-32.47, -3.53]
Dufour 2006	-41	36	12	-35	55	15	0.6%	-6.00 [-40.49, 28.49]
Ekhlasi 2016	-3.7	6.8	15	4.1	3.4	15	50.6%	-7.80 [-11.65, -3.95]
Magosso 2013	-5.9	13.3	30	-0.6	13.3	34	17.6%	-5.30 [-11.83, 1.23]
Pervez 2017	-13	17.82	31	-3.62	18	33	9.7%	-9.38 [-18.16, -0.60]
Sanyal 2010	-37	52	78	-20.1	48	74	3.0%	-16.90 [-32.80, -1.00]
Total (95% CI)			**232**			**233**	**100.0%**	**-7.37 [-10.11, -4.64]**

Heterogeneity: Tau² = 0.00; Chi² = 5.77, df = 6 (P = 0.45); I² = 0%
Test for overall effect: Z = 5.28 (P < 0.00001)



In the other trials, including the Ekhlasi *et al*. 2016 paper that provided the most statistical weight [50.6%, if you look at the column entitled **Weight** in the figure], ALT/AST levels were toward the high end of normal. Thus, although the magnitude of effect is clearly greatest in participants with NASH, vitamin E supplementation may provide a moderate benefit to a fatty liver before full-blown NASH.

Interesting Finding

When have you *ever* heard someone talk about the tocotrienols when it comes to vitamin E? So, let' s recap a bit on this fascinating fat-soluble vitamin. Vitamin E is comprised of four tocopherols [alpha (α), beta (β), gamma (γ) and delta (δ)], and four tocotrienols [alpha (α), beta (β), gamma (γ) and delta (δ)]. α-tocopherol has historically assumed pride-of-place in the vitamin E world, due to it being the predominant form in circulation, primarily carried by VLDL and LDL [5]. However, in brain autopsy studies of dementia, higher levels of γ-tocopherol are associated with less amyloid-beta plaque and neurofibrillary tangles, as we covered in a previous Deepdive, so there is more going on in the vitamin E picture than α-tocopherol alone.

For the tocotrienols, that 'more going on' appears to relate to their chemical difference from tocopherols, which means that tocotrienols are more efficiently absorbed into tissues with saturated fatty acid tissue layers, such as the liver [6,7]. Isoform for isoform, tocotrienols exhibit greater anti-oxidant and anti-inflammatory effects compared to tocopherols, however, their metabolism is more rapid and they have a much shorted half-life compared to α-tocopherol [6,7].

Nevertheless, this greater potential effect on oxidative stress and inflammation means the tocotrienols have emerged as potential therapeutic agents for liver disease. Although the magnitude effect for tocotrienols was lower than that for tocopherols in the present meta-analysis, it was only based on two studies for tocotrienols, one of which used 400mg mixed tocotrienols while the other study specifically used 600mg delta(δ)-tocotrienol. Thus, we currently lack better understanding for both the optimal isoform(s) of tocotrienols and effective dose thereof in adults with liver disease, but this remains an interesting area to keep a tab open on.

Relevance

There are caveats galore, as detailed under **Cons**, above, and if the findings of the present meta-analysis were not consistent with the wider evidence, those caveats would carry more weight.

In the TONIC trial in children with NAFLD, 800IU/d natural form α-tocopherol resulted in resolution of NASH in 58% of participants compared to 28% in the placebo group, however, there was no effect on ALT in children with NAFL [the primary endpoint] [8].

In adults, the PIVENS trial showed that 800IU/d natural form α-tocopherol resulted in significant reductions in liver fat, inflammation, and ballooning, in participants with NASH [9].

The present meta-analysis thus provides a degree of confirmatory evidential synthesis of the benefits to vitamin E supplementation on markers of liver function in adults with NALFD, which show greater magnitudes of effect in adults with higher baseline ALT/AST values and with a diagnosis of NASH. This is consistent with the recommendations for vitamin E supplementation in adults with NASH made by the American Association for the Study of Liver Diseases [4].

However, the present study does not close the book on the application of vitamin E in liver disease, but rather leaves some open questions, in particular related to the application in NAFL without confirmed NASH, and in relation to the potential therapeutic efficacy of tocotrienols.

Application to Practice

For the clinicians among you working in this area, vitamin E supplementation – which at this point in terms of strength of evidence does appear to be natural form α-tocopherol – is supported as an adjuvant option for the treatment of NAFLD, specifically NASH [(4)].

However, there is no evidence to recommend broad supplementation for general hepatic health or prevention. In that context of general health and nutrients, a food-first approach emphasising dietary intake is always going to be the default. If further research continues to illuminate the tocotrienols, that may be another reason to keep cereal grains – barley and oats especially – in the diet, to complement the tocopherol-rich plant oils and seeds.

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