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TITLE: The role of micronutrients in the pathogenesis of alcohol related liver disease

RUNNING TITLE: Micronutrients and alcohol related liver disease

AUTHORS

Dr Ruairidh Nicoll1, Konstantinos Gerasimidis2, Professor Ewan Forrest1

1 Department of Gastroenterology, Glasgow Royal Infirmary, 84 Castle Street, Glasgow, G4 0SF

2 Department of Human Nutrition, School of Medicine, College of Medicine, Veterinary and Life Sciences, University of Glasgow, New Lister Building, Glasgow Royal Infirmary, Glasgow G31 2ER, UK

CORRESPONDING AUTHOR

Dr Ruairidh Nicoll
Gastroenterology Registrar
Glasgow Royal Infirmary
84 Castle Street
Glasgow
G4 0SF

Email: Ruairidh.nicoll2@nhs.scot
Telephone: 07704951521

KEYWORDS

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ABSTRACT

Aims

Chronic alcohol consumption may result in liver-injury and chronic liver disease but other factors are likely to influence disease progression. Malnutrition, specifically micronutrient deficiency, is frequently associated with both alcohol use disorder and chronic liver disease. We hypothesise that micronutrient deficiencies may affect the progression of liver disease in this population.

Methods

Systematic integrative review of the medical literature. Electronic search of MEDLINE 1950-2021. Studies investigating role of any micronutrient in the acceleration of alcohol related liver injury in humans or animals. Studies which specifically related to alcoholic hepatitis were excluded. Outcomes were extracted and recorded in tabulated form and discussed narratively.

Results

We identified 46 studies investigating the role of micronutrient deficiencies in the pathogenesis of alcohol-related liver disease. Specific micronutrients which were identified included folic acid or related B vitamins (n= 9 studies), Vitamin D (n=9 studies), magnesium (n= 8 studies), zinc (n= 8 studies), and selenium (n=12 including one systematic review). Observational evidence suggests a potential role of magnesium deficiency in accelerating alcohol-related liver injury with weak or negative evidence for other micronutrients.

Conclusions

Magnesium deficiency may increase the risk of alcohol-related liver injury and adverse liver outcomes. However, currently, there is insufficient evidence to support magnesium supplementation except for clinically relevant magnesium deficiency. Long-term prospective cohort studies assessing the impact of micronutrients on liver disease progression in patients with alcohol
use disorder are lacking and may help determine whether there is a causal role for micronutrient deficiencies in alcohol-related liver injury.

246 words

(250 words max)
Micronutrients play a key role in various metabolic processes relevant to alcohol-related liver injury. Basic science and observational human studies support a role for magnesium deficiency in the exaggeration of alcohol-related liver injury. Evidence that other micronutrients affect alcohol related liver injury is weak.

44 words

(50 words max)
Introduction

Alcohol misuse is one of the commonest causes of chronic liver disease (Blachier, 2013) which is a major cause of morbidity and mortality worldwide (Ferrari, 2014). Liver steatosis occurs early in the majority of individuals with alcohol use disorder (AUD), and may then progress to steatohepatitis, fibrosis and cirrhosis. Cirrhosis can be asymptomatic but with persistent liver injury, hepatocellular function declines and portal hypertension occurs leading to decompensated liver cirrhosis and a sharp increase in morbidity and mortality (Seitz, 2018). Severe steatohepatitis, with or without underlying cirrhosis, may result in the clinical syndrome of acute alcoholic hepatitis which has a high short-term mortality (Veryan, 2020). Unfortunately, there are no specific interventions to prevent alcohol related liver injury other than alcohol abstinence.

The progression from steatosis towards cirrhosis is not predictable at an individual level. Whilst almost all individuals who consume over 40 grams alcohol per day will develop steatosis, only 10-35% will develop steatohepatitis and 8-20% will develop cirrhosis (Seitz, 2018). This suggests that factors other than the absolute alcohol dose play an important role in determining which patients progress from simple steatosis to fibro-inflammatory injury. Known risk factors for ArLD include female sex and ethnicity (Wagnerberger, 2008). Several specific genes, including PNPLA3, TM6SF2, MBOAT7, MARC1 and HNRNPUL1 have also been linked with ArLD (Stickel 2011, Buch 2015, Salameh 2015, Innes 2020). Co-existent liver disease such as chronic Hepatitis C virus infection and non-alcoholic fatty liver disease (NAFLD) also increase the risk of disease progression (Seitz, 2018). Nevertheless, these factors probably do not explain all the variability in liver outcomes for those who consume harmful quantities of alcohol.

One factor which may be relevant to the progression of ArLD is malnutrition. Whilst there is no universally accepted definition of malnutrition, it is probably best thought of as a multidimensional concept including abnormalities of dietary intake, macronutrient status (e.g. depleted body fat and
protein reserves), and micronutrient status which have adverse health outcomes (Dasarathy, 2016). The concept that malnutrition may have a role in ArLD pathogenesis has been argued previously (Best, 1949). However, the idea that malnutrition independently drives liver disease in those with alcohol use disorders was largely refuted in the 1970s when diet-controlled studies in rats by Lieber and DiCarli showed that it was alcohol consumption which caused liver disease (Lieber, 1972; Mezey, 1983). Nevertheless, malnutrition could act as a risk factor for alcohol related liver injury by causing more profound fibro-inflammatory liver injury.

Since these early studies, our understanding of alcohol related liver injury has advanced. The precise mechanisms resulting in alcohol related liver injury are not fully understood, but several components are now thought to be important, including altered lipid metabolism, oxidative stress, epigenetic changes and disruption of the gut barrier and gut microbiome. A detailed review of ArLD pathogenesis is beyond the scope of this review and can be found elsewhere (Seitz, 2018).

Interestingly, several components of malnutrition have the potential to augment these disease mechanisms at a biochemical level making malnutrition a plausible risk factor for alcohol-related liver injury.

Individuals with AUD are more likely to be malnourished compared to the general population. Anthropometric measures of nutritional status, including body mass, body-mass index (BMI) (Dickson, 1983; Goldsmith, 1983; Estruch 1993; Aparisi, 2001), body fat (Koehn, 1993), and muscle bulk (Dickson, 1983; Goldsmith, 1983; Koehn, 1993; Estruch, 1993; Knudsen, 2014; Song 2016) are all reduced compared to the general population. Individuals who misuse alcohol have reduced levels of B Vitamins (de la Vega, 2001), as well as Vitamins A, C, D and E (Hurt, 1981; Sangwan, 2000; Chang 2001; Lee 2012; Bergheim 2003; Teriaky, 2017). They are also more likely to be deficient in magnesium, zinc, selenium (Sullivan, 1979; Cook 1991) and possibly other trace elements including copper and manganese (Sullivan 1979; Manari, 2003).
Several mechanisms may explain the negative impact of alcohol consumption on nutritional state. Firstly, the energy yield of alcohol is considerable at approximately 7kCal/gram which is greater than that of carbohydrate (4kCal/g) and not much less than fat (9kCal/g) (Cederbaum, 2012; Barve, 2017). Alcohol therefore has the potential to displace other sources of nutrition from the diet. Secondly, alcohol has toxic effects on the gastrointestinal tract that impair digestion and absorption (Rajendram, 2005; McClain, 2011). Long-term alcohol misuse may result in chronic pancreatitis and exocrine insufficiency, resulting in impaired digestion of macronutrients (Voiosu, 2020). In the small bowel, alcohol use is associated with shortening of the villi and a reduction in the area for nutrient absorption, increased mucosal permeability and leucocyte infiltration, as well as overgrowth of the small bowel bacterial flora (Rajendram, 2005; McClain, 2011). Thirdly, alcohol use also leads to an increase in basal metabolic rate (Klesges, 1994). Lastly, alcohol has direct toxic effects on skeletal muscle, the main reserve of protein, due to upregulation of myostatin which inhibits protein synthesis (Thapaliya, 2014).

In this review, we investigate whether micronutrient deficiencies associated with AUD may accelerate alcohol related liver injury. If correct, this would enhance our understanding of ArLD pathogenesis and allow health providers to identify individuals at greater risk of alcohol related liver injury and target alcohol related interventions at those at greatest risk. In addition, it might suggest specific nutritional interventions that could be studied to minimise risk of disease progression. In this semi-systematic integrative review, we aim to extract and summarise the literature investigating the role of micronutrient deficiencies on the progression of ArLD. The aim is to provide a broad overview of this topic, highlight existing evidence and direct future research goals.

**Methods**

We performed a systematic integrative review of the role of nutritional factors in progression of ArLD. Inclusion criteria were those studies available in English which examined the association between any nutritional factor (dietary intake, overall macronutrient state, and specific...
micronutrients) and the severity and/or progression of ArLD. We included observational studies in humans as well as controlled animal studies. Relevant intervention studies of specific micronutrients were included for context and where relevant to the discussion. Studies which related specifically to the distinct syndrome of alcoholic hepatitis were excluded as the authors considered this to be a distinct syndrome within the umbrella of ArLD which could theoretically have a unique relationship with nutrition.

An electronic search of MEDLINE was performed for primary studies using Web of Science between 1950 and 2021. The initial search was performed in April 2020 and updated through to May 2021. Search terms used were as follows: (1) ‘alcohol’ or ‘ethanol’ PLUS (2) ‘nutrition’ or ‘malnutrition’ or ‘*nutrient’ PLUS (3) ‘liver’ or ‘hepat*’. After the initial search identified several specific micronutrients (folic acid, Vitamin D, zinc, magnesium and selenium) as areas of specific study, this search was repeated with line (2) replaced by ‘folic acid’, ‘folate’, ‘Vitamin D’, ‘magnesium’, ‘zinc’ and ‘selenium’. Relevant review articles were also extracted and examined for further potential primary studies.

Titles and abstracts were screened by one author (RN). Full text articles due for consideration were examined by both authors (RN, EF) prior to inclusion. Any disagreements were resolved by discussion and consensus. Data from relevant studies was extracted and summarised narratively. Outcomes of interest included the association between a micronutrient and any one of liver-related mortality, progressive liver disease demonstrated by liver biopsy (or other marker of liver disease), or liver injury as demonstrated by liver enzyme derangement. Due to the diverse nature of the topic and study heterogeneity there was no role for a meta-analysis.

**Results**

The initial electronic search, later updated to 3rd June 2021, identified 1538 study titles after exclusion of duplicate studies (Figure 1). After review of titles for relevance, this was reduced to 227 studies whose abstracts were then reviewed. Of these, 58 potentially relevant review articles were
identified. Following abstract review, 46 primary studies were identified for full-text review and included in final review. Specific nutritional factors which were identified including folic acid (or related B vitamins) (n= 9 studies including one systematic review), Vitamin D (n=9 studies), magnesium (n= 8 studies), zinc (n= 8 studies), and selenium (n=12 including one systematic review).

**The role of Folic acid and other B Vitamins in the methionine cycle**

Folic acid deficiency is a frequent complication of chronic heavy alcohol use owing to reduced dietary intake, impaired absorption across the small intestine, reduced uptake into the liver and increased urinary excretion (Herbert, 1963; Medici, 2013).

Folic acid is required for the conversion of homocysteine to methionine (with Vitamin B12 acting as a cofactor). Betaine can also be used to convert homocysteine to methionine via a different biochemical pathway. Methionine is in turn is converted into S-adenosylmethionine (SAM), the methyl-donor for all methylation reactions involving DNA (Medici, 2013). Therefore, deficiency of folic acid, or other related B vitamins such as Vitamin B12, or Betaine have the potential to promote hypomethylation of DNA and result in altered gene expression. Glutathione (GSH), a key antioxidant, is also produced from homocysteine through the transulfuration pathway. SAM and Vitamin B6 (Pyridoxine) are both required for this process. (Halsted, 2004; Medici, 2013). Therefore, folic acid or Vitamin B6 deficiency have the potential to promote oxidative stress.

We identified a total of nine studies investigating the role of folic acid or related B Vitamins in ArLD including one systematic review (Table 1). Various animal studies have investigated the effects of folic acid and related B Vitamins on alcohol induced liver injury with mixed results. Folic acid deficiency combined with alcohol feeding has been shown to increase homocysteine levels, reduce levels of SAM and glutathione and also result in DNA hypomethylation (Halsted, 2002; Rajdl, 2016). Folic acid deficiency has also been shown to increase alcohol related liver injury through activation of CYP 2E1 and increased endoplasmic reticulum stress (Esfandiari, 2005). Supplementation with folic acid and betaine have been shown to attenuate homocysteineamia (Rajdl, 2016) and betaine
supplementation can been shown to attenuate alcohol-induced steatosis (Kharbanda, 2007).

Disturbed Vitamin B6 metabolism does occur in patients with ArLD (Ma, 2020; Medici, 2010) but Vitamin 6 deficiency does not worsen alcohol-induced liver injury in animals (Diehl, 1987) and supplementation with Vitamin B6 or B12 does not reduced alcohol induced hyperhomocysteinaemia (Rajdl, 2016).

Human intervention studies have shown no benefit of SAM supplementation on liver-related outcomes. In a Cochrane systematic review (Rambaldi, 2006) of 8 studies involving a total of 330 patients, SAM supplementation was not found to impact on liver or non-liver related mortality, or on complications of liver disease. A further randomised controlled trial of SAM supplementation in patients alcohol related liver disease published after this review also demonstrated no difference between groups in clinical or biochemical parameters, or in liver histology (Medici, 2011). Overall, despite plausible biochemical mechanisms there is a lack of compelling evidence that B Vitamin deficiency plays a major role in worsening alcohol-related liver injury.

**Vitamin D**

Vitamin D is well known for its role in calcium homeostasis and bone health (Veldurthy, 2016). In addition, newer evidence suggests Vitamin D has a role in regulating immunity and may also have anti-oxidant effects (Li, 2014; Zhong, 2014; Zhu, 2017).

Animal studies have shown that Vitamin D deficiency exaggerates alcohol-related oxidative stress and liver injury (Zhang, 2019; Hu, 2020) and that treatment with Vitamin D can protect against alcohol related oxidative stress and liver injury (Blngul, 2021). It has also been demonstrated that Vitamin D can protect against alcohol-induced gut epithelial barrier dysfunction (Chen, 2015).
However, Vitamin D deficiency appears to protect against acute alcoholic steatosis through modifying hepatic lipid metabolism (Hu, 2019).

In humans, Vitamin D levels inversely correlate with increased severity of chronic liver disease (Putz-Bankuti, 2012), and an observational Danish cohort study found an inverse correlation between Vitamin D levels and the development of liver disease (Skaaby, 2014). However, an observational study of over 12,000 healthy adults in the United States found a positive correlation between Vitamin D levels and serum alanine transferase (ALT) (Shehata, 2016). A small non-controlled intervention study of Vitamin D supplementation in 50 adults with alcohol related liver cirrhosis who were abstinent with alcohol demonstrated an improvement in Child-Pugh score (Savic, 2018). However, with no control group it’s not possible to comment on causality and the observed change could have simply occurred as a result of prolonged abstinence leading to recompensation. Overall, the significance of a Vitamin D deficiency in the exaggeration of alcohol related liver injury in humans remains unclear.

Magnessium

Magnesium functions as a co-factor for over 200 enzymes and is required for cell aerobic respiration, thiamine activation, protein and nucleic acid synthesis, cell cycling, and cytokine synthesis (Romani, 2008). Magnesium deficiency has the potential to promote oxidative stress by increasing iron accumulation and the production of peroxinitrate (Johnson, 2001).

We identified eight studies linking magnesium deficiency with accelerated alcohol related liver injury (Table 1). In animal models of ArLD, magnesium supplementation led to higher levels of antioxidants, reduced oxidative stress, lowered serum transaminases and less severe steatohepatitis (Markiewicz-Gorka, 2011) and magnesium deficiency has been associated with more severe liver fibrosis (Rayssiguier, 1985). In observation studies of humans with AUD, magnesium deficiency
correlated with elevated serum transaminases (Gala, 2019) and worse liver damage (Riche, 1986). In a large cohort study of over 13,000 individuals, increased dietary magnesium intake correlated with reduced mortality from liver disease, particularly in those who consumed alcohol (Wu, 2017). Similar findings were identified in a large US cohort of 4166 adults where magnesium levels inversely correlated with the presence of liver fibrosis as measured using transient elastography (Tao, 2021). Two randomised controlled trials of magnesium supplementation in humans with alcohol dependence have shown that magnesium supplementation resulted in lower serum transaminases (Gullestad, 1992; Poikolainen, 2008). However, both studies were small and of short duration (six and eight weeks respectively) so impact on long-term outcomes is unclear.

These findings, in particular the two large population cohort studies are promising and suggest a potential role for magnesium deficiency as a risk factor for alcohol-related liver injury. They need to be taken with some caution, as alcohol use disorders are themselves associated with magnesium deficiency and therefore magnesium deficiency could be acting as a surrogate for overall alcohol consumption. Placebo controlled studies of magnesium supplementation in patients with alcohol use disorders would be helpful in ascertaining whether magnesium deficiency has an independent role here.

**Zinc**

Zinc is a cofactor for alcohol dehydrogenase, as well as being important for cell signal transduction and gene expression (Das, 1984; Indo, 1985; Kang, 2005) and deficiency may increase the amount of ethanol metabolised via CYP 2E1 and produce oxidative stress (Das, 1984; Albano, 1996). Zinc deficiency has also been linked with lipid peroxidation, reduced fatty acid beta-oxidation, impaired autophagy and hepatocyte apoptosis, reduced TNF-alpha production and an increase in circulating endotoxin (Sullivan, 1980; Gurtovenko, 1988; Caballeria, 1997; Pathak, 2002; Kojima-Yuasa, 2003; Lambert, 2003; Zhou, 2004; Zhou, 2005; Kang, 2005; Zhou 2010; Zhong, 2015; Sun, 2016; Liuzzi, 2018).
We found a total of eight studies investigating the role of zinc deficiency in ArLD (Table 1). In animals, zinc deficiency was found to correlate with higher levels of steatosis and fibrosis (Conde-Martel, 1992) zinc supplementation also improved steatosis (Kang, 2009), hepatocyte necrosis (Zhou, 2002) and reduced collagen deposition (Gimenez, 1992). The protective effects of zinc were associated with reduced lipid peroxidation and increased levels of antioxidants (Zhou, 2002). We found three human observational studies investigating the role of zinc deficiency in alcohol related liver injury. In humans with AUD but not cirrhosis, zinc deficiency is associated with reduced levels of antioxidant enzymes and liver damage (Saribal, 2019) and higher serum transaminases (Vatsalya, 2018). However, the literature is not consistent with one comparative study of patients with alcohol related liver cirrhosis and healthy controls finding that serum zinc levels did not independently correlate with the pro-fibrotic factor FGF-19 (Prystupa, 2017). Therefore, the role of zinc deficiency in ArLD pathogenesis remains unclear.

Selenium

Selenium is an important for effective functioning of the antioxidant enzyme glutathione peroxidase (GPx) (Oner, 1995) and deficiency therefore has the potential to affect levels of antioxidants (Schisler, 1988; Rua, 2014).

We found a total of twelve studies including one systematic review investigating the role of selenium in ArLD (Table 1). Animal studies have found that selenium supplementation improves glutathione peroxidase activity and protects against alcohol induced oxidative stress, lipid peroxidation and liver injury, leading to lower serum transaminases and less severe steatohepatitis (Kong, 1996; Lee, 2001; Sivaram, 2003; Markiewicz-Gorka, 2011; Wang, 2013; Ozkol, 2017; Fu, 2018; Adali, 2019). However, this effect has not been demonstrated universally (Oner, 1995). In humans with ArLD, selenium deficiency is associated with higher serum transaminases (Tanner, 1986) and increased mortality (Gonzalez-Reimers, 2008). However, a Cochrane systematic review of antioxidants in ArLD, including
eight trials of selenium supplementation, found no beneficial effect on all-cause or liver-specific mortality (Bjelakovic, 2010). Therefore, selenium deficiency is unlikely to be a significant independent risk factor for more severe alcohol-related liver injury.

Discussion

In this review, we highlight several micronutrients which have been studied as potential risk factors for alcohol-related liver injury including, folic acid, Vitamin D, magnesium, zinc and selenium. We found some supportive animal studies for each of these micronutrients. However, only with regards to magnesium was there more convincing human cohort studies to support a role in alcohol-related liver injury. At present though, there is no evidence from randomised controlled studies to suggest that supplementing magnesium actually reduces the risk of developing significant liver disease. We also found evidence from randomised controlled trials in humans that targeting folic acid and selenium does not alter alcohol-related liver outcomes.

There is reasonable evidence from population studies that magnesium deficiency is associated with an increased risk of alcohol-related liver injury. The nature of this relationship remains unclear. Whilst animal models have suggested a pathophysiological role through increased oxidative stress, it remains possible that magnesium deficiency may be serving as a surrogate marker of overall alcohol consumption. Whilst we have some evidence from supplementation studies that suggest magnesium may ameliorate liver injury there is no evidence that administering magnesium to patients with alcohol use disorders for the purpose of preventing liver injury is effective with regards to important outcomes such as the development of clinically significant liver fibrosis, cirrhosis or decompensation in those with established liver cirrhosis. Therefore, administering magnesium should be reserved for the treatment of otherwise clinically significant magnesium deficiency only.

Additionally, that there is a recurrent pattern, possibly with the exception of magnesium, of animal studies showing promise for the role of a specific micronutrient in the pathogenesis of ArLD followed by a failure of human studies to substantiate the claim. This finding may result from animal models
not accurately representing ArLD in humans either through extremes of micronutrient deficiency, alcohol excess or simply different mechanisms of alcohol-related liver injury between humans and animals. There is certainly a general lack of compelling evidence that deficiency of any single micronutrient has a clinically significant effect on progression of ArLD in humans. However, it should be remembered that most patients with AUD who are malnourished are likely to be deficient in multiple micronutrients. Therefore, a number of individually small and clinically insignificant effects may be compounded together in individuals with multiple micronutrient deficiencies and act together to produce an overall clinically significant effect. It is our view that this may explain why highly controlled positive studies in animals do not translate to positive studies in humans where deficiencies may be less extreme but multiple. Large prospective cohort studies of patients which assess the role of multiple aspects of nutrition together, including relevant micronutrients and macronutrient status would allow for a more sophisticated understanding of combined and relative contributions of different nutritional factors on the progression of ArLD. A clearer idea of which micronutrient deficiencies predict progression of liver disease would facilitate the development of interventions more likely to be effective. This may involve targeting multiple nutrients simultaneously.

In summary, emerging evidence suggests a possible role for selective magnesium deficiency in accelerating alcohol-related liver injury although there is currently no role for supplementation except in the treatment of magnesium deficiency. Prospective cohort studies in humans with AUD would help determine the individual and combined effects of different nutritional components to the pathogenesis of alcohol-related liver disease.

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REFERENCES


