Nutrition, nonalcoholic fatty liver disease and the microbiome: recent progress in the field

Miriam B. Vos\textsuperscript{a,b}
\textsuperscript{a}Emory University, Atlanta, Georgia, USA
\textsuperscript{b}Children’s Healthcare of Atlanta, Atlanta, Georgia, USA

Abstract

**Purpose of review**—Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease worldwide and it has overlapping pathogenesis with diabetes and cardiovascular disease. Reviewed here are recent advances in understanding the contribution of diet and selected nutrients to NAFLD.

**Recent findings**—To understand the effect of diet, the microbiome must be considered because it is the interface of diet and the liver. Early studies suggest that the characteristic of the microbiota is altered in NAFLD. Fructose is a lipogenic carbohydrate that contributes to insulin resistance, hypertriglyceridemia and appears to be associated with the severity of NAFLD. Fructose absorption and malabsorption may alter the microbiota and which could be mediating effects on the liver. Lipids also have potent microbiome interactions and could contribute to the benefit of diets emphasizing lipid changes. Several new studies demonstrate that the Mediterranean diet and ‘lifestyle change’ are effective in modestly improving NAFLD. A new study of lifestyle in children showed simultaneous improvement in CVD risk measurements and hepatic steatosis.

**Summary**—Current data supports limiting sugar in the diet and ‘lifestyle change’ as a first-line treatment for NAFLD; however, the benefits from these appear to be modest. The effects of diet on the liver are mediated through the microbiome and expansion of research in this area is needed.

**Keywords**
diet; fructose; microbiome; nonalcoholic steatohepatitis

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a chronic liver disease that typically occurs in the setting of excess weight gain, particularly increased visceral adiposity. NAFLD, the umbrella term, includes a spectrum of disease including simple steatosis, nonalcoholic steatohepatitis (NASH) that includes steatosis along with inflammation and fibrosis, and end-stage disease, cirrhosis. NAFLD is clinically silent until the advanced stages and is typically identified during screening or incidentally during evaluation for another medical issue. NAFLD is increasing in prevalence, despite the recent leveling off of the obesity prevalence. It is the most common chronic liver disease worldwide and is responsible for substantial...
morbidity and mortality, largely from associated conditions rather than cirrhosis. NAFLD increases the risk of cardiovascular events eight-fold over traditional risk factors, increases risk of new onset type II diabetes three-fold and is associated with increased cancers [1].

Because of the vast clinical burden of NAFLD and its overlapping pathogenesis with CVD and diabetes, there is a huge amount of ongoing research and knowledge is advancing rapidly. Nutrition research in NAFLD is complicated by methodologic challenges and to date there is not a clear single dietary approach for patients with NAFLD. This review examines literature from the past year on the role of diet in NAFLD. Some of the studies reviewed here are helpful for clinical practice but also delineate the challenges that lie ahead. A brief review of new research on the microbiome is covered first because understanding the microbiome has complicated nutrition science but also may be the key.

MICROBIOME: INTERFACE OF DIET AND LIVER

‘We are what we eat’ is the old adage but the modern version might be ‘we are what our bacteria eat.’ Nonculture-dependent techniques have evolved the understanding of the microbiome and its role in health and disease. The gut microbiota process dietary components and the products become signaling molecules that direct gene regulation in the human host to favor storage in adipose tissue (reviewed in [2]). Bacterial patterns appear to vary between obese humans and lean humans; increased Firmicutes and decreased Bacteroidetes is one identified pattern, although data on phyla variations remains conflicting [2]. Those with obesity-related diseases have less diversity in their microbiota. Gut microbiota composition has a strong link to food patterns and can vary by fat content of the diet, plant polysaccharide content, calorie content and ‘Western’ diets [2]. The fate of a person’s bacterial flora could be set very early. A recent article published in Nature demonstrated that early exposure to antibiotics changed the colonic microbiota and the body adiposity [3]. In low-level antibiotic exposed infant mice, the cecal levels of short-chain fatty acids were increased and there was up-regulation of gene pathways related to fatty acid metabolism, lipogenesis and triglyceride synthesis in the liver. Calorie intake was not increased in the heavier, antibiotic exposed mice but their feces contained fewer calories suggesting efficient calorie extraction as a mechanism for the adiposity gain. This mechanism is important because it highlights a common environmental exposure that could have sustained effects in the response to diet.

The liver is the natural recipient of the messages from the gut microbiota because blood from the intestines is received in the sinusoids of the liver through the portal system. Growing evidence links NAFLD and the microbiome. The earliest data suggested that patients with NASH have small intestine bacterial overgrowth more often compared with healthy controls and have increased intestinal permeability with altered tight junctions [4,5]. A high-fat diet is known to cause hepatic steatosis although studies of saturated, monounsaturated and polyunsaturated have had varying results. de Wit et al. [6] hypothesized that changes in the small intestine could contribute to the effects of a high-fat diet. They studied three different high-fat diets in mice: palm oil, olive oil and safflower oil. Those on palm oil had the greatest increase in body weight, hepatic triglyceride and increase in plasma insulin and this was associated with a decrease in microbiota diversity. The most pronounced difference was increased Firmicutes to Bacteroides ratio; however, the high-fat olive oil group also gained weight but did not have the increased Firmicutes to Bacteroides ratio. The researchers hypothesized that the changes were from increased fat delivery in the distal portions of the intestine because the fecal fat content was the highest in the high-fat-palm oil group. Furthermore, microarray analysis on distal small intestine tissue showed pronounced changes in genes related to lipid metabolism. The high-fat-palm oil group did not have increased energy intake or absorption, rather they must have had a difference in
energy dissipation. These findings are important because they demonstrate how diet quality can induce changes in the microbial signaling from the gut and alter calorie utilization. Studies like this one could help explain the highly variable response to diet seen in both research studies and in ‘real life’.

Thus far, the studies of the microbiota and human NAFLD are primarily association. Mouzaki et al. [7] evaluated stool in adults and found decreased abundance of Bacteroides in those with NASH compared with simple steatosis and healthy control participants. Another group examined colonic microbiome and volatile organic compounds in NAFLD patients compared with healthy controls [8]. Again significant differences were found including overabundance of lactobacillus species and both over and underrepresentation of various phyla of Firmicutes. Perhaps more importantly, they found increased fecal ester compounds, a product of microbes, in the NAFLD group. Zha et al. examined endogenous alcohol, another product of the microbiota. Under normal conditions, blood alcohol goes up after the intake of alcohol free food and this is thought to be from the production by the intestinal microbiota. They tested levels in children with NASH compared with healthy and obese children and found significantly increased blood alcohol levels that were associated with variation in average phylum distribution of gut microbiota. These studies are confirming a very important concept, namely that the effect of diet may be mediated through the intestinal microbiome whose products flow into the liver and from there are transmitted to the rest of the body contributing to disease (or health). The understanding of this complexity is changing the nature of nutrition science particularly related to liver diseases.

**Fructose and NAFLD**

Fructose is a nutrient that has been much studied in NAFLD and newer studies have improved methodology as well as consideration of a microbiome effect. Fructose consumption is elevated today compared with past decades [9,10] and the major sources high fructose corn syrup and cane sugar are ubiquitous in the diet, particularly of children and adolescents. A collection of studies have shown that in a hypercaloric setting, fructose induces hypertriglyceridemia, visceral adiposity and decreases insulin sensitivity (recently reviewed [11]). The role of fructose in NAFLD appears to be two-fold; one of inducing TG production via de-novo lipogenesis and resulting in hyperlipidemia and 2nd, contributing to inflammation resulting in insulin resistance, hepatic inflammation and fibrosis. Early work in mice suggested a role for the microbiota influencing response to fructose because fructose increased endotoxin in portal venous blood and administration of antibiotics decreased both endotoxin and hepatic steatosis associated with fructose [12]. Bergheim et al. [13,14] have continued to examine the mechanisms of this and demonstrated fructose increases intestinal translocation of bacterial endotoxin and subsequent activation of Kupffer cells through TLR dependent mechanisms. They and others have shown that prebiotics and probiotics in mice protect from fructose-induced hepatic steatosis [15,16]. Kavanagh [17] studied a group of monkeys on a long-term high fructose diet. In the monkeys, the caloric burden of fructose appeared to make a difference; the monkeys on calorie-controlled high fructose diet developed hepatic inflammation, endotoxemia and microbial translocation whereas monkeys on an ad-libitum high fructose diet developed hepatic steatosis and had increased incidence of diabetes [17]. In humans, fructose is absorbed via facilitated transport primarily through Glut 5 and malabsorption is common at levels above 50 g, well above the US average intake [18]. Malabsorbed fructose in the small intestine would pass on to the colon and interact with bacteria there. Genetic and adaptive variation in Glut 5 as well as the microbiota could help explain tolerance or lack of tolerance of fructose. Walker et al. [19] examined fructose mal-absorption and found that obese African American participants had both less liver steatosis on imaging as well as greater fructose malabsorption.
In NAFLD, fibrosis severity is associated with fructose [20] and children with NAFLD have greater postprandial hypertriglyceridemia with fructose compared with healthy children [21]. Reducing fructose in the diet of children with NAFLD improves ALT [22] and oxidized LDL [23]. New clinical studies confirm that fructose can increase existing steatosis. An overfeeding study of overweight adults who consumed an extra 1000 kcal/day from candy, pineapple juice, sugar sweetened soft drinks and sports drinks showed that over 3 weeks, liver fat increased by 27% and during a subsequent 6-month hypocaloric diet declined by a similar amount [24]. In animal models, both glucose and fructose can induce steatosis [12] and this appears to be true in humans as well. Thirty-two centrally overweight men participated in a crossover study comparing high glucose and high fructose beverages provided as 25% of estimated caloric needs in a research study provided diet [25]. In a hypercaloric condition over 2 weeks, both the glucose group and the fructose group gained weight and hepatic fat increased by a mean of 2 and 1.7%, respectively. This study reinforces the clinical application of sugar research that glucose cannot be ignored and one should limit added sugars, not just fructose. It also mirrors animal data that has long demonstrated that hypercaloric glucose diets also generate intrahepatic lipid in susceptible mice [12]. Richard Johnson’s group recently published a fascinating group of experiments demonstrating that glucose generation of intrahepatic lipid may be mediated viafructose because in settings of excess, fructose can be generated by the polyol pathway. Aldose reductase metabolizes glucose into sorbitol and from there sorbitol dehydrogenase converts it to fructose. They used knockout mice to demonstrate that without fructokinase to metabolize fructose, high glucose does not result in a fatty liver. Their insights could help explain the well established ‘high carbohydrate’ effects seen from glucose sources in the diet. For NAFLD, the collective data continues to support that sugar should be limited. The important research questions that remain include how much sugar is well tolerated, does source matter (solid food?, drink? naturally occurring?), and the multitude of possible roles of the microbiome.

**Dietary patterns**

Growing evidence supports the role of dietary patterns rather than single nutrient changes. Although this is challenging to study, many groups have undertaken this approach. A study of dietary quality found that the overall components were different in NAFLD compared to those without NAFLD [27]. This is a theme that pervades the literature, specifically although it is difficult to select ‘the nutrient’ that is responsible, collections of dietary habits appear to make a substantial difference to health. Given the newer data on diet and the microbiome, patterns of diet may be acting via effects in the intestine. A dietary change can shift the microbiota over time although it reverts when the diet is stopped. For example, a 6-week energy-restricted high-protein diet in 49 obese or overweight participants resulted in increased diversity of species in the gut and decreased adiposity compared with baseline [28]. However, the microbial diversity reverted to baseline after the diet was stopped and there was less improvement in inflammatory factors in those with less diversity at baseline suggesting a divergence of inflammation from the microbiota effect [28].

For NAFLD, a diet that appears to be beneficial is the Mediterranean diet. The Mediterranean diet has long been advocated for atherosclerosis and many studies evaluated effect on CVD risk [29]. The benefit is thought to be from the MUFA in olive oil, although animal studies testing derivatized MUFA have not found the same benefits. Olive oil is a plant extract and other bioactive components could be responsible [29]. Furthermore, little data is available regarding possible effects of the Mediterranean diet on the microbiota. Two new studies examined Mediterranean diet and NAFLD. Kontogianni et al. [30] evaluated 73 newly diagnosed patients who met the clinical definition of NAFLD and compared them with 58 healthy adults. They assessed diet using food frequency questionnaires and assigned

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a Mediterranean diet score. They did not find a difference in diet score between healthy and NAFLD; however, those with NASH had significantly lower scores compared with steatosis alone. Diet is difficult to test and perhaps the best way to investigate it is to provide the diet. Ryan et al. [31] undertook this ambitious task in a randomized, cross-over study comparing a Mediterranean diet to a diet they termed ‘low-fat, high carbohydrate’ based on Australian Heart Foundation Association recommendations and the American Heart Association. They studied 12 adults with biopsy proven NAFLD and measured hepatic steatosis using magnetic resonance spectroscopy, the most precise noninvasive measurement of liver fat available. After 6 weeks of MD, steatosis declined by an average of 5% when compared with minimal change in the low-fat diet. Insulin sensitivity measured by oral glucose tolerance tests also improved but interestingly there was not much change in TG or HDL.

Some of benefit in NAFLD of the Mediterranean diet could be because of the fat source. Omega-3 fatty acids are long-chain poly-unsaturated fatty acids that are high in seafood and also green leafy vegetables, some seeds, nuts and legumes and consumption is typically low in typical ‘Western’ diets. Diets low in omega-3 fatty acids may increase production of proinflammatory factors and impair hepatic regulation of lipids. Previous intervention studies in humans have demonstrated improvement in hepatic steatosis from seal oil and from olive oil, both high in omega-3 fatty acids [32]. Recent studies have confirmed a low intake in children with NAFLD. Papandreou et al. [33] examined 82 obese children in Greece and assessed NAFLD using ultrasound. Total carbohydrates were increased in NAFLD and omega-3 fatty acids were decreased. A cross-sectional study of US children with NAFLD showed only 10% consumed the recommended 8 oz of fish per week and just 12% consumed the recommended 200 mg of long-chain fatty acids per day [34]. Low fish and omega-3 fatty acid intake correlated with the severity of inflammation on liver biopsy. There is a new RCT underway testing fish oil versus sunflower oil in children with NAFLD so more information will be forthcoming [35]. Given the animal data reviewed above, omega-3 fatty acids in the diet could exert their influence via the microbiome. There is evidence that fish oil high in omega-3 fatty acids affect lipid metabolism by increasing the expression of cholesterol and bile acid transporters in the intestine as well as the liver [36].

‘Lifestyle modification,’ another global approach to nutrition changes for patients continues to show benefit, in at least improving ALT in NAFLD. Diet and exercise combination have been established as the first-line therapy for obesity, and are accepted as such for NAFLD in both children and adults. However, the evidence that lifestyle changes (and which ones) improve histology is lacking. Eckard et al. [37] tackled this question in a 6-month randomized, controlled trial of lifestyle modification including four subgroups: standard of care, low fat diet (20% fat), moderate fat (30%) with moderate exercise and moderate exercise alone. Standard-of-care participants attended one healthy eating class whereas the low fat groups had more extensive nutrition advice. The results were very interesting; all four groups had modest improvements in disease severity as measured by standardized scores of histologic features on biopsy (NAFLD activity score) whereas the two low fat groups showed greater improvements. This was despite no weight loss although over half the individuals initially lost weight and then regained it. Clinically, lifestyle change appears to work. A busy pediatric liver group recently reported their outcomes and overall showed improved transaminases, LDL, cholesterol and BMI [38]. In a larger, 1-year lifestyle intervention program, 120 children with NAFLD demonstrated improvements in both cardiovascular endpoints as well as decreases in hepatic steatosis measured by MRI [39]. The cohort on average improved BMI, improved brachial flow-mediated dilation measurements, TG, HOMA-IR, ALT and decreased hepatic steatosis by approximately 50%. This is one of the first studies to demonstrate reversibility in subclinical markers of atherosclerosis in NAFLD and lends strong support for lifestyle education as the first line therapy, particularly in children. They also measured cIMT and did not see a change. The
lifestyle program as reported does not appear particularly intensive although it is unclear how many visits the education portion entailed and further information would be needed to understand if this could be implemented in routine clinical practice.

**CONCLUSION**

In summary, the progress in the area of nutrition and NAFLD is rapid and this remains an area of extremely high interest. The advent of studies that include examination of the microbiome in NAFLD is one of the most exciting areas in NAFLD and nutrition research. The microbiome is the interface between the diet and the liver and appears to modify or even direct the effects of nutrients. Fructose is a nutrient with substantial new evidence of its role in NAFLD in the past year. The data support fructose (or practically speaking ‘sugar’) reduction as a clinically valuable approach for patients. Clinical trials improving diet pattern and increasing exercise have been recommended for CVD, obesity and diabetes and these ‘lifestyle changes’ remain a first-line therapy for NAFLD. However, the level of improvements seen even with a reduction in BMI is modest and unlikely to ‘cure’ the disease. Future studies are needed to examine the microbiota in ‘dietary change’ studies for NAFLD because this may shed light on diversity and durability of response to diet as well as insight into mechanisms.

**REFERENCES AND RECOMMENDED READING**

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


21. Jin R, Le NA, Liu S, et al. Children with NAFLD are more sensitive to the adverse metabolic effects of fructose beverages than children without NAFLD. J Clin Endocrinol Metab. 2012; 97:E1008–E1098. This study demonstrates that children with NAFLD have a greater acute increase in triglycerides from fructose compared with children without NAFLD.


25. Johnston RD, Stephenson MC, Crossland H, et al. No difference between high-fructose and high-glucose diets on liver triacylglycerol or biochemistry in healthy overweight men. Gastroenterology. 2013; 145:1016–1025. This study confirms in humans a finding seen in many
animal models of high-sugar diets, namely that both hypercaloric glucose diets and hypercaloric fructose containing diets can increase hepatic steatosis. [PubMed: 23872500]

26. Lanaspa MA, Ishimoto T, Li N, et al. Endogenous fructose production and metabolism in the liver contributes to the development of metabolic syndrome. Nat Commun. 2013; 4:2434. This important article describes a series of experiments showing that glucose is converted to fructose in the liver and blocking this conversion protects against the development of hepatic steatosis. [PubMed: 24022321]


31. Ryan MC, Itsiopoulos C, Thodis T, et al. The Mediterranean diet improves hepatic steatosis and insulin sensitivity in individuals with nonalcoholic fatty liver disease. J Hepatol. 2013; 59:138–143. This is a small, high quality randomized, 6-week cross-over trial of the Mediterranean diet compared to a low fat, high carbohydrate diet. The study demonstrated improvement in hepatic steatosis and insulin sensitivity with the provided Mediterranean diet food. [PubMed: 23485520]


37. Eckard C, Cole R, Lockwood J, et al. Prospective histopathologic evaluation of lifestyle modification in nonalcoholic fatty liver disease: a randomized trial. Ther Adv Gastroenterol. 2013; 6:249–259. This is a RCT of lifestyle changes for NAFLD and is one of the only lifestyle studies that includes a histologic outcome. The study proves that lifestyle modification improves NAFLD and NASH.


KEY POINTS

- The microbiome is the interface between diet and the liver and modifies dietary effects.
- Fructose and ‘sugar’ reduction are a valuable clinical approach for patients with NAFLD.
- Almost any dietary approach or ‘lifestyle’ change appears to show some benefit and can be recommended as a first step for treating NAFLD.
- The best diet for NAFLD is not yet known.