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TOPIC HIGHLIGHT

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Nutrition and physical activity in NAFLD: An overview of the epidemiological evidence

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Abstract

Nonalcoholic fatty liver disease (NAFLD) has been recognized as a major health burden. The high prevalence of NAFLD is probably due to the contemporary epidemics of obesity, unhealthy dietary pattern, and sedentary lifestyle. The efficacy and safety profile of pharmacotherapy in the treatment of NAFLD remains uncertain and obesity is strongly associated with hepatic steatosis; therefore, the first line of treatment is lifestyle modification. The usual management of NAFLD includes gradual weight reduction and increased physical activity (PA) leading to an improvement in serum liver enzymes, reduced hepatic fatty infiltration, and, in some cases, a reduced degree of hepatic inflammation and fibrosis. Nutrition has been demonstrated to be associated with NAFLD and Non-alcoholic steatohepatitis (NASH) in both animals and humans, and thus serves as a major route of prevention and treatment. However, most human studies are observational and retrospective, allowing limited inference about causal associations. Large prospective studies and clinical trials are now needed to establish a causal relationship. Based on available data, patients should optimally achieve a 5%-10% weight reduction. Setting realistic goals is essential for long-term successful lifestyle modification and more effort must be devoted to informing NAFLD patients of the health benefits of even a modest weight reduction. Furthermore, all NAFLD patients, whether obese or of normal weight, should be informed that a healthy diet has benefits beyond weight reduction. They should be advised to reduce saturated/trans fat and increase polyunsaturated fat, with special emphasize on omega-3 fatty acids. They should reduce added sugar to its minimum, try to avoid soft drinks containing sugar, including fruit juices that contain a lot of fructose, and increase their fiber intake. For the heavy meat eaters, especially those of red and processed meats, less meat and increased fish intake should be recommended. Minimizing fast food intake will also help maintain a healthy diet. PA should be integrated into behavioral therapy in NAFLD, as even small gains in PA and fitness may have significant health benefits. Potentially therapeutic dietary supplements are vitamin E and vitamin D, but both warrant further research. Unbalanced nutrition is not only strongly associated with NAFLD, but is also a risk factor that a large portion of the population is exposed to. Therefore, it is important to identify dietary patterns that will serve as modifiable risk factors for the prevention of NAFLD and its complications.

Key words: Nonalcoholic fatty liver disease; Nutrition; Physical activity; Weight reduction; Fat; Carbohydrates; Soft drinks; Nutrients

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NUTRITION AND OBESITY IN NAFLD PATHOGENESIS

Nonalcoholic fatty liver disease (NAFLD), which develops in the absence of alcohol abuse, has been recognized as a major health burden. The clinical implications of NAFLD are derived mostly from its common occurrence in the general population and its potential to progress to cirrhosis and liver failure[1-3]. Estimates suggest that about 20% to 30% of adults in developed countries have excess fat accumulation in the liver[4-8], 50% among people with diabetes, and about 80% in the obese and morbidly obese[6,9,10].

The high prevalence of NAFLD in Western countries is probably due to the contemporary epidemics of obesity and associated metabolic complications. Obesity, type 2 diabetes, and hyperlipidemia are recognized as risk factors for NAFLD[5,11-14]. Insulin resistance is frequently detected in patients with NAFLD, as it is in those without obesity and diabetes[14-19]. An increasing number of patients have been described with normal body mass index (BMI), although these individuals may have central adiposity and occult insulin resistance[17,18,20,21]. Moreover, epidemiological studies[22-24] indicate that this unique group of normal weight patients is characterized by an unhealthy dietary composition, as will be discussed later.

The efficacy and safety profile of pharmacotherapy in the treatment of NAFLD remains uncertain[25], and obesity is strongly associated with hepatic steatosis[26]; therefore, the first line of treatment is lifestyle modification. The usual management of NAFLD includes gradual weight reduction and increased physical activity, leading to an improvement in serum liver enzymes, reduced hepatic fatty infiltration, and, in some cases, a reduced degree of hepatic inflammation and fibrosis[27-33]. However, most studies did not include repeated liver biopsy, and thus histological improvement could not be determined.

Although research is emerging, it remains uncertain whether diets that are enriched with certain types of food or nutrients are more likely to cause fatty liver than other types of diets[26]. In light of the difficulty in reducing weight and maintaining the weight reduction in the long term[34], changing dietary composition without necessarily reducing calorific intake may offer a more realistic and feasible alternative to treat NAFLD patients. Therefore, exploring the association between specific nutrients and dietary composition and NAFLD is extremely important.

This review discusses the existing epidemiological evidence for the association between human NAFLD and dietary composition, weight reduction, and physical activity.

THE AMOUNT AND TYPE OF DIETARY FAT

Total dietary fat vs carbohydrates

There are three major sources for the increased triglyceride deposition in the liver: excessive influx of free fatty acids (FFA) from endogenous fat depots, increased *de novo* hepatic lipogenesis, and exogenous-nutritional fat. Recent human studies suggest that a significant fraction of fatty acids are taken up by the liver during the postprandial period[35,36]. Furthermore, NAFLD patients may exhibit alterations in postprandial hepatic lipid metabolism. In a study using an oral fat load test in 15 Nonalcoholic steatohepatitis (NASH) patients and 15 controls, total and very low density lipoproteins triglyceride in postprandial plasma were higher in NASH compared with controls, and postprandial plasma Apo B48 and Apo B100 responses in NASH were flat. This suggested an increased hepatic uptake of triglycerides in the postprandial period combined with reduced hepatic secretion of VLDL, which may promote liver steatosis[37].

Multiple studies in animals have documented that a high-fat diet rapidly induces hepatic steatosis[38-40], but data in humans are scarce.

The association between total dietary fat and hepatic fat content has been directly tested in humans by placing 10 obese women on two successive two-wk isocaloric diets, which contained either 16% or 56% of energy from fat, in randomized order using a crossover design and assessing the liver fat by proton spectroscopy. Liver fat decreased by 20% on the low-fat diet and increased by 35% on the high-fat diet. The changes in liver fat were paralleled by changes in fasting serum insulin concentrations. Importantly, these changes were independent of body weight, which did not change during the study[36].

In another study, 74 morbidly obese patients (90% of them with NAFLD) undergoing bariatric surgery underwent a preoperative dietary evaluation using a 24-h food recall. Food intake was compared to liver histopathology from biopsies obtained during surgery. There were no significant associations between total calorific intake or protein intake and either steatosis, fibrosis, or inflammation. However, higher carbohydrate intake (above 54% of calories) was associated with significantly higher odds of inflammation, while higher fat intake was associated with significantly lower odds of inflammation. However, this study was unable to discern any differences in specific dietary fat composition, perhaps due to insufficient power or misclassification of fat intake based on a single 24-h recall and, importantly, did not differentiate between simple *vs* complex carbohydrates[41]. The association with carbohydrates is supported and sharpened by a study from Japan comparing dietary habits between 28 patients with NASH and 18 with simple steatosis, indicating an excess intake of carbohydrates, especially of sweets and not cereals, in the NASH group[42].

Although the results appear conflicting, it would seem reasonable to say that over-consumption of either fat or carbohydrates is not recommended, and eventually all mac-

ronutrients should be consumed according to the accepted recommendations (e.g. those of the American Heart Association), as part of a balanced diet. Furthermore, as will be demonstrated later in this review, the specific subtypes of fat (saturated *vs* unsaturated and its subgroups) and carbohydrates (complex *vs* simple and its subgroups) may be more important than their total amount.

Type of dietary fat and other nutrients

In contrast to cardiovascular and metabolic diseases, there is little epidemiological evidence that the type of dietary fat is associated with fatty liver[5]. A small sample size study, but with meticulous dietary assessment based on 7-d alimentary record, evaluated 25 normal-weight NASH patients compared with age-, gender-, and BMI-matched controls. The dietary intake of NASH patients was richer in saturated fat and cholesterol, and was poorer in polyunsaturated fat, fiber, ascorbic acid, and tocopherol[37]. These results are supported by another study in which the ratio of polyunsaturated/saturated fatty acid intake in both the NASH and fatty liver patients was lower than the ratio in randomly selected controls[42].

The type of dietary fat has also been demonstrated to be associated with oxidative stress markers in non-alcoholic steatohepatitis. Analyzing dietary intake obtained by a food frequency questionnaire in 43 NASH patients and 33 healthy controls, a negative correlation was found with total and saturated fat intake, and with the ratio of reduced Plasma glutathione/oxidized glutathione, indicating an impaired glutathione metabolism and suggesting a pro-oxidant effect. Conversely, a positive correlation was found with carbohydrates, fiber, monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA), specifically n-3 polyunsaturated fatty acid (n-3 PUFA)[43].

Different types of fats can have a protective effect in NAFLD. The most established one is the n-3 PUFA. Experimental studies have shown that diets enriched with n-3 PUFA increase insulin sensitivity in rats[44], reduce intra-hepatic triglyceride content, and ameliorate steatohepatitis[45,46].

Two observational studies provide evidence of a lower consumption of omega-3 PUFA among NAFLD patients. The first is a case-control study of 45 NASH patients compared with a sample of 856 controls, matched for sex and age[47]. Diet history assessed by a food frequency questionnaire (FFQ) demonstrated a significantly higher intake of n-6 fatty acids and a higher n-6/n-3 ratio among NASH patients. These results suggest that the quality and combination of fat intake may be more relevant than its isolated amount; an excessive amount of n-6 fatty acids could be implicated in promoting necro-inflammation[47].

The second study was a cross sectional study in 349 volunteers from the general population. Diet history assessed by an FFQ demonstrated higher meat intake ($P < 0.001$) and a tendency ($P = 0.056$) to a lower intake of fish rich in omega-3 in NAFLD patients. n-6 fatty acids are abundant in meat; therefore, these data suggest a higher intake of n-6/n-3 ratio in NAFLD patients (Zelber-Sagi, Nitzan-Kaluski, Goldsmith, Webb, Blendis, Halpern *et al*, 2007).

Two pilot clinical trials support the protective role of omega-3 PUFA in NAFLD. The first was a nonrandomized open-label controlled trial that assessed the effect of a one-year n-3 PUFA supplementation (containing both eicosapentaenoic acid-EPA and docosahexaenoic acid-DHA) at a dose of 1000 mg/d in 42 NAFLD patients versus 14 patients that refused the treatment and were analysed as controls. PUFA supplementation significantly decreased serum liver enzymes (ALT, AST, and GGT) and reduced liver fat (as measured by ultrasonography) as compared to controls[48]. The second study was a non-controlled trial in 23 NASH patients that were supplemented with 2700 mg/d of EPA for one year. Serum ALT levels were significantly improved. Seven of the 23 patients underwent post-treatment liver biopsy, which showed improvement of hepatic steatosis and fibrosis, hepatocyte ballooning, and lobular inflammation in six patients[49]. In both trials, body weight remained unchanged.

There are two types of fat, trans fatty acids (TFA) and MUFA, which so far have not been tested or demonstrated to be associated with human NAFLD. However, based on their association with related diseases, such as diabetes and cardiovascular disease, should be considered in the nutritional recommendations of NAFLD.

Trans fatty acids

Little is known about the role of TFA in promoting liver injury in NAFLD. The association between TFA and increased risk of developing insulin resistance[50] and coronary heart disease by raising LDL cholesterol levels, lowering HDL cholesterol levels, raising triglyceride levels, and increasing CRP[51] suggest that it may be involved in NAFLD pathogenesis.

Compared with PUFA and saturated fatty acids-fed mice, TFA-fed mice had impaired glucose tolerance, characterized by greater homeostasis model assessment (HOMA), and NASH-like lesions due to greater hepatic lipogenesis[52]. In another experiment, the effect of a combination of features of a western lifestyle was tested. In mice fed TFA in a high-fat diet, high-fructose corn syrup, and interventions designed to promote sedentary behavior, isocaloric replacement of TFA with lard indicated that TFA played a major role in promoting hepatic steatosis and injury[53]. Therefore, the role of TFA in human NAFLD needs to be evaluated, which presents a challenge to nutritional epidemiologists, as information on TFA content in food is unknown in many cases.

Monounsaturated fatty acids

n-9 oleic acid is the most prevalent MUFA in the diet, and olive oil is one of its major sources (other sources are nuts and avocado). MUFA has been demonstrated to have a favorable effect on the lipid profile, with a reduction in both the LDL and total cholesterol to HDL ratio[54]. A meta-analysis of randomized, crossover trials comparing low-saturated-fat, high-carbohydrate diets or high-MUFA diets

in patients with type 2 diabetes revealed that high-MUFA diets improve lipoprotein profiles as well as glycemic control. High MUFA diets reduce fasting plasma triacylglycerol and VLDL-cholesterol concentrations by 19% and 22%, respectively, and cause a modest increase in HDL-cholesterol concentrations without adversely affecting LDL-cholesterol concentrations[55].

In rats with an MCD diet, olive oil was demonstrated to decrease the accumulation of triglycerides in the liver by 30% compared with the only MCD diet group. The serum triglycerides increase was 10% lower in the MCD diet + olive oil group compared with the MCD group. Olive oil improved insulin resistance, increased the release of Triglycerides from the liver, and decreased the flux of FFAs from peripheral adipose tissue back to the liver[56]. In rats, treatment with a balanced diet rich in olive oil contributed to the recovery of the liver from hepatic steatosis[57]. Olive oil, in contrast to polyunsaturated oils, was demonstrated to protect against the development of fibrosis[58].

However, it has not been demonstrated that NAFLD patients eat less MUFA as compared to controls[23,24,59], and the role of MUFA or olive oil in human NAFLD is yet to be demonstrated.

Cholesterol

With regard to cholesterol, results from observational studies have been conflicting. Some studies did not demonstrate different dietary intakes of cholesterol between NAFLD patients and controls[47,59]. However, Musso *et al*[23] did demonstrate a higher cholesterol consumption among normal weight NASH patients *vs* BMI matched controls. A recent study supported the role of dietary cholesterol in NAFLD. In this study, 12 normal weight NAFLD patients were compared to 44 obese NAFLD patients. A characteristic feature was that dietary cholesterol intake was significantly higher, while the intake of polyunsaturated fatty acids (PUFAs) was significantly lower, in the nonobese group. Similar differences were noted in comparison to 15 healthy non-obese controls. Therefore, this altered cholesterol and PUFA intake may be associated with the development of NAFLD in non-obese patients[24].

In addition, studies using non-obese animal models have confirmed that a hypercholesterol diet can induce NASH[60]. An increase in dietary cholesterol is suggested to induce *de novo* fatty acid synthesis in hepatocytes *via* the LXRa-SREBP-1c pathway[24].

In conclusion, studies testing the association with different types of dietary fats in normal weight NAFLD patients may clarify nutritional composition as a direct risk factor.

THE ASSOCIATION BETWEEN ADDED SUGAR AND SOFT DRINKS CONSUMPTION AND NAFLD

Soft drinks are a leading source of artificially added sugar in the world[61].

In recent decades, intake of sugar-sweetened beverages has increased around the globe[62]. Recent data (2005-2006) show that children and adults in the United States consume about 172 and 175 kcal/d, respectively, per capita from sugar-sweetened beverages[63]. The consumption of sugar-sweetened beverages has been linked to risks for obesity, diabetes, metabolic syndrome, fatty liver, and heart disease, possibly by providing excess calories and large amounts of rapidly absorbable sugars[59,64-69]. In a recently published health policy report, taxation of sugar-sweetened soft drinks has been proposed as a means of reducing the intake of these beverages and thereby lowering disease burden and health care costs[62].

A sucrose-rich diet increases the hepatic synthesis of triglycerides. Rats and humans that are fed either sucrose- or fructose enriched diets develop fatty livers[65,70]. Therefore, it is reasonable to suggest that NAFLD patients should limit their fructose consumption[71]. In addition, cola soft drinks contain caramel coloring, which is rich in advanced glycation end products (AGEs), which can increase insulin resistance and inflammation[61].

In recent years, several studies have been published on the association between soft drinks consumption and NAFLD, demonstrating a positive association[22,59,72,73]. The first was a cross-sectional study of a sub-sample (n = 375) of the Israeli National Health and Nutrition Survey (MABAT 1999-2001). A semi-quantitative food-frequency questionnaire was administered and showed that NAFLD patients have a higher intake of soft drinks. Moreover, the higher intake of soft drinks was associated with an increased risk of NAFLD, independently of age, gender, BMI, and total calories[59].

In a study on 31 normal weight NAFLD patients with no obvious classic risk factors and 30 healthy controls matched for gender and age, it was found that NAFLD patients consume significantly higher amounts of added sugar and that most of it (43%) comes from soft drinks and juices, compared to only 8% in the controls[22]. Another study by the same group demonstrated similar results; 80% of patients with NAFLD had excessive soft drinks intake (> 500 cc/d) as compared to 17% in controls[73].

In a recent study, the consumption of fructose containing beverages was compared in NAFLD patients and controls matched for gender, age, and BMI. It was demonstrated that consumption of fructose in patients with NAFLD was two-fold higher compared to matched controls[72].

Recently, a large-scale study of 427 NAFLD patients expanded the understanding of the hepatic damage that may be related to over-consumption of fructose-containing beverages. After controlling for age, sex, BMI, and total calorie intake, daily fructose-containing drinks consumption was significantly associated with higher fibrosis stage (OR = 3.2, 1.4-7.4 95% CI for $\geq$ 7 servings *vs* < 7 per week) in both younger and older age groups, and a lower steatosis grade, but only in the older group of patients[74].

Thus, these studies identified an important modifiable risk factor. Physicians and dietitians should routinely in-

clude questions regarding soft-drink consumption as part of the patient's history and advise patients to avoid it.

WESTERN DIETARY PATTERN AND FAST FOOD

Examination of overall dietary patterns would more closely parallel the real world, where people eat meals consisting of a variety of foods with complex combinations of nutrients that may be interactive or synergistic[75]. The studies presented above regarding dietary composition usually provide indications for several nutrients or foods that characterize the dietary intake of NAFLD patients, and may be looked at as an unhealthy pattern or western dietary pattern. This pattern seems to include overconsumption of fructose and soft drinks[22,59,72], lower consumption of fiber[47], overconsumption of meat[59] or saturated fat and cholesterol[23,24], lower consumption of fish or omega-3 fatty acids[47,59] or PUFA[24], and lower consumption of some vitamins[23], which may indicate a below the recommended consumption of vegetables and an unbalanced diet in general.

Fast-food consumption has strong positive associations with weight gain and insulin resistance in humans. In the CARDIA study, a 15-year prospective follow up of 3031 young adults, those with frequent (more than twice a week) visits to fast-food restaurants gained an extra 4.5 kg of bodyweight and had a two-fold greater increase in insulin resistance compared to participants with infrequent (less than once a week) fast-food consumption[76]. Furthermore, feeding experimental animals with the "cafeteria diet" (a feeding regimen similar to fast food) leads to liver damage[53].

What happens if we do the same experiment in humans? 18 healthy, young students were put on a fast food diet that included at least two fast food meals a day for four weeks. They increased their caloric intake and body weight and their HOMA values doubled. Hepatic triglyceride content increased, as did serum ALT levels. After the intervention started, 11 out of 15 with normal ALT levels at baseline had elevated ALT levels at one week; eight had persistent elevation during the intervention; and two had persistent elevation even at six months follow up. Thus, in clinical evaluations of subjects with elevated ALT levels, medical history should include not only questions about alcohol and soft drinks intake, but should also explore whether recent excessive intake of fast food has occurred[77].

Potential mechanisms of hepatotoxicity are high energy density & portion size, high fat & saturated fat, high refined carbohydrate, low fiber, high fructose corn syrup, caramel coloring, red meat, industrially produced trans fatty acids, promoting free fatty acid overflow to the liver and local inflammation[78].

WEIGHT REDUCTION

Throughout the past decades, three types of trials have dealt with weight reduction in NAFLD. Historically, the first ones, in the 1960s, tested very low calorie diets (VLCD) leading to drastic weight reduction. These diets were based on fasting and formulas and did not discuss behavioral therapy at all. Thereafter, a second wave of trials explored a more balanced diet, sometimes combined with physical activity, only mildly referring to behavioral therapy and sometimes with short-term follow up. More recently, structured balanced diets combined with a detailed behavioral therapy program aiming at a long-term lifestyle modification have been evaluated.

Examples of the first generation are small sample trials from the 1960s[79] and 1970s[80] that included fasting or very low calorie diet (about 500 kcal), leading to drastic weight reduction. Steatosis was reduced in all patients, but liver damage, as indicated by fibrosis and focal necrosis, was observed in some patients during the acute weight loss. In a later study from 1991, Andersen *et al*[81] provided 41 morbidly obese patients with a 400 kcal formula-based diet, again leading to improved steatosis. However, 24% developed slight portal inflammation ($P = 0.039$) or slight portal fibrosis ($P = 0.063$). This study helps in setting the upper limit for the rate of weight reduction in NAFLD patients, as none of the patients who lost less than 1.6 kg/wk developed fibrosis. Interestingly, liver biochemistry improved regardless of the histological changes[81]. In another VLCD study, a weight reduction of greater than or equal to 10% resulted in normalized abnormal hepatic test results in most patients; however, liver biopsies were not obtained[82].

Two small sample size studies tested the effect of a balanced diet and gradual weight reduction on liver histology. Ueno *et al*[83] demonstrated significant reductions in hepatic steatosis after only three months on treatment. Hepatic inflammation and fibrosis also improved, although not significantly, probably because of the short follow up. The one year long term trial included behavioral therapy, with regular meetings with a dietitian and group sessions, and weekly food records. Nine out of 15 patients, who lost an average of 7% of their body weight, had an improved NASH score, and the remaining six, who had no weight loss, had stable scores[84].

The next study is one of a few RCT's testing weight reduction in NAFLD. In a 48-wk intervention, 32 NASH patients were randomized to receive intensive lifestyle intervention or basic education about healthy lifestyle (controls). A moderate, balanced diet was combined with moderate-intensity activities, with particular emphasis on walking with pedometers. Classical behavioral strategies were also extensively applied: self-monitoring of eating and exercise, stimulus control techniques, problem solving *etc*. NASH histological activity score (NAS) improved significantly in the treatment arm in comparison with the control group. Participants who achieved weight loss of > 7% compared with those who lost less than 7%, had significant improvements in steatosis, lobular inflammation, ballooning injury, and NAS[85]. In the Orlistat trial by Harrison *et al*[86], a somewhat bigger weight reduction of at least 9% was necessary to achieve sig-

nificant improvement in NAS, although 5% reduction was sufficient for improving steatosis. Recently, another RCT tested the effect of a 12-mo intensive lifestyle intervention on hepatic steatosis in a specific subgroup of patients with type 2 diabetes. The intervention included a moderate calorific restriction plus increased physical activity and weekly meetings, whereas the control group received only general information on nutrition and physical activity. After 12 mo, participants assigned to the intensive intervention, as compared to controls, lost more weight (-8.5% *vs* -0.05%; $P < 0.01$) and had a greater decline in steatosis measured by H-MRS (-50.8% *vs* -22.8%; $P = 0.04$). The intervention was also beneficial in prevention of NAFLD, as 26% of controls *vs* 3% of intervention participants, without NAFLD at baseline, developed NAFLD at 12 mo[87].

Three recent, relatively large sample size studies addressed the effect of diet, provided in different settings, on ALT levels[88-90]. In the trial by Suzuki *et al*[89] 348 male subjects with elevated ALT were recruited from annual health checkups, and were given health care instructions using customized brochures and then followed at health checkups three times a year. At one year follow up, all subjects achieving ⩾ 5% weight reduction showed improvement in serum ALT and 136 subjects had ALT normalization. In the second trial 152 patients with elevated liver enzymes were randomized to either a moderate (6 sessions/10 wk) or low-intensity (3 sessions/4 wk) lifestyle counseling intervention or control group. Reduction in liver enzymes was greatest in the moderate-intensity intervention group and least in the control group, in parallel to the proportion of subjects achieving weight loss[88]. In the third trial, with a smaller sample size, 67 patients with NAFLD were enrolled into a 6-mo home-based lifestyle modification intervention, which included monthly visits with a physician and nutritional counselling every three months. At six months, there were significant improvements in terms of body weight, liver/spleen ratio, and liver enzymes. This study's flaw was a large attrition rate, with only 22 patients (33%) completing the 6-month intervention[90], perhaps indicating that patients require a more intensive follow up.

The challenge in demonstrating the therapeutic efficacy of weight reduction in NAFLD has been the lack of liver histology as an outcome in most studies, or on the other hand, the limited sample size and statistical power whenever liver biopsy is undertaken because of its invasive nature. Liver biopsy is necessary for the evaluation of therapeutic effects beyond reduction in ALT and regression of steatosis on imaging (or only disappearance of steatosis when simple ultrasound is applied).

This is especially important because certain diets may seem beneficial according to reduction of liver enzymes, while actually leading to liver damage that can only be observed by liver biopsy. Future advances in identification and validation of non-invasive methods for hepatic fibrosis, inflammation, and quantification of steatosis should help determine if weight reduction is effective for treating all the features of NAFLD, at which stages of the disease should weight reduction be introduced, the optimal weight reduction rate and nutrient composition. According to research so far, although mostly small sample size trials have been performed, the results are consistent and indicate that weight reduction can be considered as an established treatment.

PHYSICAL ACTIVITY

From the perspective of NAFLD patients, weekly or daily performance of walking, swimming, or cycling might seem as simple as jumping of the cliff.

As with diet, low long-term compliance is also the rule for increased physical activity: on average 20% after two years follow up[91]. Despite the difficulties, increased physical activity (PA) is highly beneficial. Indeed, PA has been shown to reduce the risk of T2DM, insulin resistance, hypertension, dyslipidemia, impaired fasting glucose (IFG), and the metabolic syndrome[92-95]. This indicates that PA could play a role in the treatment of patients with NAFLD.

Several observational studies indicated an inverse association between reported leisure time PA, or cardiorespiratory fitness, and the prevalence of NAFLD. In a large-scale study ($n = 349$) of the general population, the NAFLD group engaged in less reported leisure time PA, including total, aerobic, and resistance. Engaging in any kind of PA remained significant after adjusting for insulin resistance and circulating adiponectin plus nutritional factors, but not BMI. Only the association with resistance PA remained significant with further adjustment for BMI[96]. A large-scale study ($n = 218$ men) demonstrated an inverse association between fitness categories and the prevalence of NAFLD, regardless of BMI[97]. In a small study on 37 NAFLD patients with liver biopsy, there was a lower cardiorespiratory fitness among patients with higher NAFLD activity score and NASH versus no NASH[98].

The beneficial effect of exercise is supported by recent clinical trials. The first[99] included 141 patients with suspected NAFLD based on abnormal liver enzymes and exclusion of other causes of liver disease who were randomized to either the intervention arms (three months physical activity counseling delivered at three intensity levels) or a control arm. Patients who increased their PA by ⩾ 60 min per week ($n = 85$) significantly reduced their weight (-2.4 kg on average), HOMA, and all liver enzymes. Importantly, these improvements were independent of the change in weight. These results are supported by a previous pilot trial demonstrating that moderate intensity aerobic exercise helped to normalize ALT levels in 65 NASH patients receiving moderately energy-restricted diet[100], although this improvement cannot be regarded as independent of weight loss. Thus, it seems that among NAFLD patients, even small increments in regular PA can improve liver enzymes; encouraging information that can be provided to patients. Another recent trial assessed the effect of short-term (four weeks) aerobic exercise training

on hepatic, blood, abdominal, and muscle lipids in 19 sedentary obese men and women using magnetic resonance imaging and proton magnetic resonance spectroscopy (1H-MRS). Four weeks of aerobic cycling exercise (three cycle sessions per week (30-45 min) significantly reduced mean hepatic triglyceride concentration by 21%, along with a 12% reduction in visceral adipose tissue volume and a 14% reduction in plasma free fatty acids. Importantly, no change in weight or dietary intake was noted, thus isolating the net effect of aerobic exercise[101].

Higher cardiorespiratory fitness at baseline may contribute to a successful hepatic outcome during lifestyle modification that includes dietary counseling and exercise. Among the parameters predicting the change in liver fat, fitness at baseline emerged as the strongest factor, independently of exercise intensity during the intervention. However, it should be remembered that cardiorespiratory fitness reflects not only recent physical activity habits, but also genetics[102].

In a recent study, 12 obese adolescents underwent a three-month resistance exercise program consisting of 2 × 1 h/wk, exercising all major muscle groups. The exercise program resulted in significant strength and lean body mass gain. Although hepatic fat content remained unchanged, hepatic insulin sensitivity increased and glucose production rate decreased, without weight loss[103]. Although aerobic exercise seems to have more extensive effects, a longer duration and/or a more intensive resistance exercise program may be required for reduction of hepatic fat content. For those who have physical limitations or low motivation that prevents them from performance of aerobic PA, resistance exercise can serve as an alternative option.

PA benefits NAFLD beyond encouraging weight reduction. Exercise alone, in the absence of any change in body weight or composition, may enhance insulin sensitivity and glucose homeostasis[104]. PA appears to result in insulin-receptor upregulation in muscle tissue and hence increased delivery of glucose and insulin to the muscles[105]. Exercise also has a beneficial effect on FFA metabolism, by enhancing whole-body lipid oxidation[106]. Hepatic triglyceride accumulation was shown to decrease with exercise intervention[107] and hepatic FFA uptake was lower in trained (endurance training) compared to untrained male subjects[108]. Similar findings were demonstrated in comparing monozygotic male twins that had a marked difference in leisure-time physical activity and aerobic fitness, where, in the absence of the confounding effects of genetic factors, the active twin had decreased hepatic FFA uptake[106].

In recent years, increasing attention has been paid to resistance training as a useful adjunctive tool of exercise[109,110]. A recent study showed that resistance training, without a concomitant weight loss diet, significantly improved insulin sensitivity and fasting glycemia and decreased abdominal fat[111]. Tsuzuku *et al*[112] demonstrated that non-instrumental resistance training, using body weight as a load, appears to be effective in decreasing visceral fat and improving metabolic profiles, without weight loss. The results of a randomized trial comparing the effect of aerobic *vs* resistance training on coronary risk factors, demonstrated that only the resistance training group showed a reduction in total body fat, with an associated increase in lean body mass[113]. A meta-analysis comparing aerobic training with weight training concluded that weight training resulted in greater increases in fat-free mass[114]. An increase in muscle mass may improve insulin sensitivity by increasing the available glucose storage area, thereby reducing the amount of insulin required to maintain a normal glucose tolerance[115].

The Centers for Disease Control and Prevention (CDC), the American Heart Association (AHA), and the Healthy People 2010 Objectives recommend adults to attain ⩾ 30 min of moderate-intensity physical activity on most, and preferably all, days of the week, or vigorous-intensity physical activity ⩾ 3 times per week for ⩾ 20 min each time. Although these recommendations have been widely publicized, only 27.7% US adults meet recommended levels of either moderate or vigorous physical activity, whereas 29.2% report no regular physical activity outside of their work[116,117]. Moreover, the prevalence of physically active adults among patients with diabetes is lower than in those without diabetes[118] and subjects with diabetes are less likely to meet physical activity recommendations[119].

In NAFLD patients, compliance may be even lower because fatigue has been demonstrated to be markedly higher in NAFLD patients compared to controls, and is associated with inactivity and excessive daytime sleepiness[120].

Apparently, the empty half of the glass - sedentary time - is by itself associated with metabolic status. Time spent sedentary, measured objectively by individually calibrated heart rate monitoring, predicted higher levels of fasting insulin, independent of the amount of time spent at moderate- and vigorous-intensity activity levels. This highlights the importance of reducing sedentary time in order to improve metabolic status, in addition to the benefits associated with a physically active lifestyle[121].

Environmental factors that discourage physical activity include an environment that encourages automobile use rather than walking (like lack of sidewalks), and that has few cues to promote activity and numerous cues that discourage activity (television, computers *etc*)[122].

POTENTIALLY THERAPEUTIC DIETARY SUPPLEMENTS

Vitamin E

Treatment with vitamin E (α-tocopherol) at high doses of 300-1000 IU/day (about 30 IU is the Recommended Dietary Allowance) has demonstrated conflicting results when leading to reduction of liver enzymes in an uncontrolled trial[123], but failed to show significant added value over lifestyle modification in controlled trials[124,125].

In a recent randomized, large long-term clinical trial, 247 NASH patients without diabetes were randomized to three arms: pioglitazone at a dose of 30 mg/d, vitamin E at a dose of 800 IU daily (84 subjects), or placebo

(83 subjects), for two years. Only vitamin E therapy, as compared with placebo, was associated with a significantly higher rate of improvement in nonalcoholic steatohepatitis (43% *vs* 19%, $P = 0.001$). Serum alanine and aspartate aminotransferase levels were reduced with vitamin E and with pioglitazone, as compared with placebo and both agents were associated with reductions in hepatic steatosis, but not with improvement in fibrosis scores[126]. Despite these promising results, treatment with high dose vitamin E should be carefully considered due to its troubling association with increased risk for hemorrhagic stroke[127] and all-cause mortality[128] in randomized controlled trials. Indeed, the authors of the study indicate that cardiovascular events occurred with equal frequency in all three study groups; however, the trial was too small to detect meaningful differences in the incidence of cardiovascular events[126].

Vitamin D

Increasing evidence suggests that vitamin D may have an important role in modifying risk for cardiometabolic outcomes, including type 2 diabetes, hypertension, and cardiovascular disease[129-131]. Serum 25(OH)D levels were demonstrated to be independently associated with both insulin sensitivity and beta-cell function among individuals at risk of type 2 diabetes[132].

Recently, serum 25(OH) Vitamin D concentrations have been shown to be associated with NAFLD[133]. Targher *et al* studied circulating 25(OH)D3 in 60 consecutive patients with biopsy-proven NAFLD, and 60 healthy controls of comparable age, sex, and BMI, and found reduced levels in those with NAFLD (51.0 ± 22 nmol/L *vs* 74.5 ± 15 nmol/L, $P < 0.001$). The differences in 25(OH)D concentrations observed between the groups were little affected by adjustment for age, sex, BMI, creatinine, calcium, HOMA-insulin resistance, and the presence of the metabolic syndrome. Furthermore, among NAFLD patients, decreased 25(OH)D concentrations were closely associated with the histological severity of hepatic steatosis, necroinflammation, and fibrosis ($P < 0.001$ for all)[133]. In a recent abstract, the association between vitamin D concentration, fatty liver, and coronary artery disease (defined as a stenosis of > 50% in at least one major coronary artery by cardiac CT) was tested in 60 patients with NAFLD compared to 30 sex, age matched healthy controls. Patients with NAFLD showed lower vitamin D concentration (13 ± 8 ng/mL *vs* 31 ± 4 ng/mL, $P < 0.001$) and severe vitamin D deficiency (< 12 ng/mL, OR 2.5, 95% CI 1.5-4.6, $P < 0.01$) predicted coronary artery disease independent of metabolic syndrome[134].

Currently, the association between vitamin D status and NAFLD and its potential therapeutic role warrants further research.

CONCLUSION

NAFLD is not only a cause of chronic liver disease and a component of the metabolic syndrome, but might also predict the tendency to develop diabetes mellitus[135-137] and has also been suggested to be associated with coronary artery disease[138-144]. In terms of public health, it will be important to detect NAFLD at a relatively young age, prior to other metabolic complications, because the treatment of NAFLD will be part of the primary prevention of type-2 diabetes and coronary artery disease.

Identifying modifiable risk factors for prevention and treatment of NAFLD is therefore important. Nutrition has been demonstrated to be associated with NAFLD and NASH in both animals[65,145] and humans[22,36,47,59,73,85], and thus serves as a major route of prevention and treatment. However, most human studies are observational and retrospective, allowing limited inference about causal associations. Furthermore, nutritional studies that rely on reported recall of diet are prone to information bias that could weaken existing associations and underestimate the contribution of certain nutrients to the pathogenesis of NAFLD. This limitation can be minimized by meticulous methods of dietary assessment (e.g. obtaining more repeated dietary recalls from each patient) to reduce measurement error, and using a larger sample size that will provide sufficient statistical power, which might uncover associations between nutrients and NAFLD. Large prospective studies and clinical trials are now needed to establish a causal relationship.

Currently no firm recommendations can be formulated, because of the lack of high quality, evidence-based data with hepatic histological outcomes. However, based on available data, patients should optimally achieve a 5%-10% weight reduction. A recent position statement on NAFLD/NASH[146] recommended on a weight loss of 7%, as proposed by International Societies on the basis of an extensive body of literature. Setting realistic goals is essential for long-term successful lifestyle modification[147,148], because obese patients tend to have unrealistic weight loss expectations (about 25%-35%) that if unmet, lead to adverse effects, such as lower satisfaction with treatment and a lower self-esteem[149]. More effort must be devoted to informing NAFLD patients of the health benefits of even a modest weight reduction, and feedback should be provided not only on weight loss, but also on individual changes in behavior and risk factors[148].

Furthermore, all NAFLD patients, whether obese or of normal weight, should be informed that a healthy diet has benefits beyond weight reduction. They should be advised to reduce saturated/trans fat and increase polyunsaturated fat with special emphasize on omega-3 fatty acids. They should reduce added sugar to its minimum, try to avoid soft drinks containing sugar (including fruit juices that contain a lot of fructose) and increase fiber intake. For the heavy meat eaters, especially those of red and processed meats, less meat and increased fish intake should be recommended. Minimizing fast food intake will also help maintain a healthy diet. Physical activity should be integrated into behavioral therapy in NAFLD, as even small gains in PA and fitness may have significant health benefits. A combination of educational, behavioral, and motivational strategies is required to help patients achieve lifestyle change[148]. Preferably, this should be

provided by multidisciplinary teams including dietitians, psychologists, and physical activity supervisors[150,151]. "Let food be your medicine" said Hippocrates; so should say more and more physicians to their NAFLD patients.

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