Nonmedicinal interventions in nonalcoholic fatty liver disease

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Potential therapeutic interventions in NAFLD/NASH must involve a good understanding of the molecular mechanisms of the disease that may reduce hepatic steatosis and the development of necro-inflammation/fibrosis by reversing defects at three levels including: reducing substrate supply for lipogenesis from excess dietary triglycerides or from excessive lipolysis and free fatty acid flux to the liver; modulating impaired glucose tolerance and type 2 diabetes mellitus, and hepatic fat accumulation ranging from simple steatosis to severe steatohepatitis (NASH). NASH is primarily a mitochondrial disease arising from the inability of the mitochondria to adapt to an oversupply of fat (3,4). In addition to the mitochondrial dysfunction and oxidative stress in NASH, the translocation of gut-derived endotoxin to the portal vein initiates liver injury (3). These processes stimulate inflammatory responses. Lipotoxicity plays a key role in the pathogenesis of NAFLD. Dysregulation of hepatic metabolism – a result of lipotoxicity signaling and inflammatory processes – illustrates the cytokine imbalance that leads to liver injury (3).

Approximately 30% to 40% of patients with NAFLD develop NASH. NASH is commonly associated with perisinusoidal and perilobular fibrosis that may progress to cirrhosis (5). Moreover, it is estimated that 10% to 30% of patients with NAFLD develop cirrhosis after 10 years (3). Additionally, NASH represents the second or third leading indication for liver transplant in North American and is projected to become the leading indication in the next 10 to 20 years (6).

Based on data from United States adult liver transplantation databases, 2004 to 2013, Wong et al (6) concluded that the number of adults with NASH awaiting liver transplant has almost tripled.

Potential therapeutic interventions in NAFLD/NASH must involve a good understanding of the molecular mechanisms of the disease that may reduce hepatic steatosis and the development of necro-inflammation/fibrosis by reversing defects at three levels including: reducing substrate supply for lipogenesis from excess dietary triglycerides or from excessive lipolysis and free fatty acid flux to the liver from insulin-resistant adipose tissue; activating key molecular steps that

Nonalcoholic fatty liver disease (NAFLD) is a chronic liver condition characterized by insulin resistance, frequently associated with impaired glucose intolerance or type 2 diabetes mellitus, and hepatic fat accumulation ranging from simple steatosis to severe steatohepatitis (NASH) (1,2). NAFLD is believed to be the most common cause of cryptogenic cirrhosis (1). The diagnosis of NAFLD is based on evidence of hepatic fatty infiltration, shown either by imaging or histology in the absence of other causes for secondary hepatic fat accumulation such as significant alcohol consumption, use of medication or presence of hereditary disorders known to produce hepatic lipidoses, or viral hepatitis B or C (1). NASH is mediated by other risk factors such as comorbid obesity and type 2 diabetes mellitus, in the presence or absence of the metabolic syndrome (1).

Morphological hallmarks of NAFLD-nonalcoholic steatohepatitis (NASH) include the severity of steatosis, hepatocyte ballooning, lobular inflammation, portal granulocytic inflammation, Mallory-Denk hyaline bodies and satellitosis (2,3). The pathology subcommittee of the Clinical Research Network for NASH designed and validated a histological feature scoring system for the full spectrum of lesions of NAFLD. This group evaluated 14 histological features and, after analysis, proposed a NAFLD activity score. This activity score includes class 1, which is simple steatosis; class 2, steatosis with lobular inflammation; class 3, the additional presence of ballooned hepatocytes; and class 4, the presence of either Mallory-Denk hyaline bodies or fibrosis. These stages were correlated with increasing severity of disease and likelihood of progression to cirrhosis (2).

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stimulate fatty acid oxidation and/or inhibit hepatic lipogenesis (ie, AMP-activated protein kinase); or by ameliorating the inflammation cascade generated by mitochondrial dysfunction from fat overload (ie, activation of Kupffer cells, local production of cytokines, induction of apoptosis, etc) (7-12).

Dietary intervention, the current standard of care for NAFLD and NASH, primarily reduces substrate supply (fat and carbohydrate overload) with modest and variable secondary improvements on hepatic molecular steps and local inflammation. The present review discusses the roles of nutrition and exercise as well as intragastic balloon placement on the evolution and progression of NAFLD/NASH, with an emphasis on changes in serum levels of adipokines (primarily adiponectin and leptin) as a result of these interventions.

DIETARY PATTERNS IN NAFLD
Several dietary components have often been found to be lacking in NAFLD patients. For example, insufficient intake of vitamin C, vitamin K, folate and omega-3 fatty acids were risk factors for NAFLD, while their increased consumption was protective (13,14). In particular, low serum 25-hydroxyvitamin D (25([OH]D)3) levels were measured among NAFLD patients compared with controls in several studies. These are summarized in Table 1 (15-19).

Serum 25(OH)D3 levels may independently predict NAFLD (18,20). The association between lower serum 25(OH)D3 levels and increased NAFLD incidence was maintained after controlling for age, sex, body mass index (BMI), creatinine, calcium, homocysteine model assessment-insulin resistance and the presence of the metabolic syndrome (15). Decreased serum 25(OH)D3 levels further predicted histological severity of hepatic steatosis, hepatocyte ballooning, necro-inflammation and fibrosis (15,17,18).

Vitamin D status was assessed as insufficient in 50.6% and as deficient in 17.3% in a sample of 156 adolescents with NAFLD. Inadequate serum 25(OH)D3 levels at 17 years of age was a risk factor for NAFLD after adjusting for sex, race, physical activity, BMI and insulin resistance (21). Vitamin D supplementation was associated with increased serum 25(OH)D3 levels, and this was accompanied by decreases in serum malondialdehyde and high-sensitive C-reactive protein (CRP) levels in a NAFLD sample (22). Inadequate intake of antioxidant vitamin C was noted in NAFLD patients (mean ± SD 84.3±43.1 mg/day) when compared with non-NAFLD control individuals (144.2±63.1 mg/day) (P=0.001). A deficient level of vitamin E was also described (5.4±1.9 mg/day in NASH versus 8.7±2.9 mg/day in control; P=0.001) (23).

Food components whose increased consumption is associated with NAFLD include fruits, nuts and grilled meat (14,23). In particular, a higher Western dietary pattern (high intakes of fast food, red meat, processed meats, full-fat dairy products, fried potatoes, refined cereals, cakes and biscuits, confectionery, soft drinks, sauces and dressings) at 14 years was associated with a greater risk for NAFLD, assessed by liver ultrasound, at 17 years (OR 1.59 [95% CI 1.17 to 2.14]; P<0.005) in a large sample of adolescents followed since birth (NAFLD was present in 15.2%). This was strongly linked to BMI at 14 years of age, such that this association was predominantly observed among obese adolescents. In contrast, a healthy dietary pattern (high in whole grains, fruit, vegetables, legumes, fish, fibre, folic acid and most micronutrients, and low in energy derived from total fat, saturated fat and refined sugar) at 14 years of age was protective against NAFLD development by 17 years of age (OR 0.63 [95% CI 0.41 to 0.96]; P=0.033) (24). In contrast, a recent cross-sectional study found similar consumption of Western diet high in fat and sodium intake and low in intakes of suboptimal micronutrients between 74 patients with biopsy-proven NAFLD and 27 healthy controls (25).

Higher BMI and waist circumference predicted a higher incidence of NAFLD (42.6%) in a sample of 82 obese children. Total carbohydrate intake was higher in NAFLD patients (P=0.001), while the intake of saturated fats was proportional to the degree of steatosis (13). Fried food consumption was associated with hepatic steatosis in a cross-sectional study of 74 overweight adolescents (P=0.04). Total fat intake (P=0.03) and the percentage of daily energy intake derived from fat (P=0.02) were associated with hepatic steatosis in this sample. Daily consumption of fibre was associated with lower visceral obesity (P=0.03) but had no influence on hepatic steatosis (26).

Overnutrition increases adipose tissue and results in insulin resistance, which favours high rates of free nonesterified fatty acid flux to the liver. This adds to the liver content of triacylglycerol, while triacylglycerol metabolites lead to a lipotoxic environment (27).

PHYSICAL ACTIVITY
Significantly lower proportions of NAFLD patients met recommendations for physical activity compared with healthy controls (P=0.02). Levels of physical activity were similar between NASH patients and subjects with simple steatosis. However, levels of physical activity were even lower among individuals who also suffer from diabetes mellitus (25,28). On the other hand, regular physical exercise decreased the risks of having elevated aspartate aminotransferase (AST) levels and of developing NAFLD in a large sample of apparently healthy, nonobese adults (29). Table 2 describes some of the results of various interventions using physical exercise in NAFLD patients (29-43).

A recent study suggests that short-term aerobic training (AT) can help reduce the risk for NAFLD progression by targeting hepatic lipid composition; this effect appears to be mediated by adiponectin (38). Physical activity was also shown to have beneficial effects on intrahepatic triglyceride content, but not on the hepatic lipoprotein kinetics (35). Both AT and resistance training led to decreases in intrahepatic lipid levels, independent of weight loss (30,38,44,45). The combination of AT and resistance training generally led to better results than either intervention alone (32). Physical exercise was also associated with lower intrahepatic lipid levels independent of weight loss in a meta-analysis (46).

A multidisciplinary program of dietary and exercise advice for developing individualized goals was found to have a positive effect on stabilizing BMI, and in improving total and low-density lipoprotein cholesterol as well as improving serum alanine aminotransferase (ALT) and AST levels for up to one-year follow-up in a prospectively
TABLE 2

<table>
<thead>
<tr>
<th>Study, sample population</th>
<th>Physical activity regimen</th>
<th>Outcome</th>
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<tbody>
<tr>
<td>Bae et al (29); 72,359 healthy adults without diabetes</td>
<td>Physical exercise (30 min/day, 3 times/week) for 3 months (n=12,967)</td>
<td>Physical exercise: lower odds of having elevated AST level (OR 0.85 [95% CI 0.74–0.99]) and ALT (OR 0.74 [95% CI 0.67–0.81])</td>
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<tr>
<td>Hallsworth et al (30); 19 sedentary adults with clinically defined NAFLD</td>
<td>RT for 8 weeks (n=11) Continued normal treatment (n=8)</td>
<td>RT: significant reduction in liver lipids (P&lt;0.05), improvements in lipid oxidation, glucose control and HOMA-IR</td>
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<tr>
<td>Bhat et al (31); 42 NAFLD patients</td>
<td>Regular AT (30 min/day for at least 5 days/week)</td>
<td>AT: decreased insulin resistance, BMI, waist circumference and ALT levels (P&lt;0.01 for all), and improved NASH scores</td>
</tr>
<tr>
<td>de Piana et al (32); 58 obese adolescents (28 with NAFLD)</td>
<td>Interdisciplinary weight-loss therapy for 1 year</td>
<td>AT + RT: improvement in body mass, BMI, fat mass, glycemia, total cholesterol and low-density lipoprotein-cholesterol in non-NAFLD patients</td>
</tr>
<tr>
<td>Malin et al (40); 121 obese NAFLD patients</td>
<td>Physical exercise (30 min/day, 3 times/week for 3 months) (n=108)</td>
<td>AT + RT: improvement in body mass, BMI, fat mass, glycemia, total cholesterol and low-density lipoprotein-cholesterol, and subcutaneous fat in NAFLD patients</td>
</tr>
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<td>Haus et al (33); 13 obese NAFLD patients</td>
<td>Moderate exercise (1 h/day) with restricted energy intake for 10 weeks</td>
<td>Training: weight loss and improvement in ultrasonographic liver steatosis, liver fat content and insulin resistance</td>
</tr>
<tr>
<td>Sullivan et al (35); 18 obese NAFLD patients</td>
<td>Exercise training (0–60 min for 5 days/week) for 16 weeks (n=12)</td>
<td>Exercise training: decreased intrahepatic triglyceride content (10.3±4.6%; P&lt;0.05), with no influence on body weight, percent body fat, and very low density lipoprotein triglyceride and apolipoprotein B-100 secretion rates</td>
</tr>
<tr>
<td>Al-Jiffri et al (36); 100 type 2 diabetes male patients with NAFLD</td>
<td>Physical training (3 times/week for 12 weeks) combined with dietary measures</td>
<td>Physical training + dietary measures: decreases in ALP (P=0.0015), ALT (P=0.0013), AST (P=0.0027), γ-GTP (P=0.0056), HOMA-IR (P=0.0083) and BMI (P=0.0094)</td>
</tr>
<tr>
<td>Bacchi et al (37); 31 sedentary adults with NAFLD and type 2 diabetes</td>
<td>Physical exercise for 4 months</td>
<td>Both AT and RT equally effectively reduced hepatic fat content (P&lt;0.001 vs baseline), led to disappearance of hepatic steatosis (defined as hepatic fat content &gt;5.56%) in almost a quarter of patients, increased insulin sensitivity during euglycemic clamp, and decreased total body fat mass, visceral adipose tissue, subfascial subcutaneous abdominal adipose tissue and hemoglobin A1c</td>
</tr>
<tr>
<td>Khoshsbaten et al (39); 90 NAFLD patients</td>
<td>Medical treatment after AT (30 min/day, 3 times/week for 3 months) (n=45)</td>
<td>Medical treatment + AT: decreased AST (41.5±2.7 at baseline vs 29±9.5 IU/L at 3 months; P=0.006) and ALT (61.1±3.6 at baseline vs 44.9±2.4 IU/L at 3 months; P=0.01)</td>
</tr>
<tr>
<td>Malin et al (40); 13 obese NAFLD patients</td>
<td>AT for 7 days (60 min/day at 85% maximum heart rate)</td>
<td>AT: reduced insulin resistance (P&lt;0.05) and circulating fetuin-A levels (P&lt;0.02)</td>
</tr>
<tr>
<td>Oh et al (41); 212 obese, middle-age men (19.8% had abnormal liver function and suspicious liver fibrosis)</td>
<td>Exercise training program without any dietary restriction for 12 weeks (n=108)</td>
<td>Exercise training increased adiponectin levels</td>
</tr>
<tr>
<td>Oh et al (42); 169 obese NAFLD patients</td>
<td>MVPA weight reduction for 12 weeks &lt;150 min/week (n=40) 150–250 min/week (n=42) ≥250 min/week (n=87)</td>
<td>Exercise training reduced serum levels of inflammation and oxidative stress markers such as ferritin and thiorbarbituric acid reactive substances in subjects with suspected liver fibrosis</td>
</tr>
<tr>
<td>Zelber-Sagi et al (43); 64 NAFLD patients without secondary liver disease</td>
<td>RT 3 times/week for 3 months (n=33) Home stretching (n=31)</td>
<td>RT reduced hepatorenal- ultrason Index (P=0.017), total, trunk and android fat, serum ferritin and total cholesterol levels</td>
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ALP Alkaline phosphatase; ALT Alanine aminotransferase; AST Aspartate aminotransferase; AT Aerobic training; BMI Body mass index; CK-18 Cytokeratin-18; γ-GTP Gamma-glutamyl transpeptidase; HOMA-IR Homeostasis model assessment-insulin resistance; MVPA Moderate to vigorous intensity physical activity; NAFLD Nonalcoholic fatty liver disease; NASH Nonalcoholic steatohepatitis; RT Resistance training; sFas Soluble Fas; vs versus
A recent study assessed the degree of weight loss necessary to improve the markers of hepatic function and insulin resistance in type 2 diabetes male patients with NAFLD. Physical training combined with dietary measures led to improvements in liver function tests and insulin resistance, while no such changes were observed in the control group receiving dietary measures alone (36). The addition of AT to medical treatment led to an improvement in serum ALT and AST levels, along with an improvement in liver echogenicity compared with medical treatment alone (39).

The severity of NASH is inversely associated with the ability to oxidize fat (50). Exercise and diet modification were shown to reduce the degree of steatosis in nonobese NAFLD patients (51). NAFLD severity was correlated with intramuscular adipose tissue content in a cross-sectional study (P<0.01), suggesting that skeletal muscle fat accumulation may influence the pathogenesis and severity of NASH (52). Changes in intramuscular adipose tissue content were correlated with changes in weight (P<0.05), BMI (P<0.05), subcutaneous fat area (P<0.01), visceral fat (P<0.05), insulin (P<0.05), homeostasis model assessment-insulin resistance (P<0.05), the quantitative insulin sensitivity check index (P<0.01), and histopathological assessments such as changes in steatosis and lobular inflammation (P<0.01 for both) (52). Improved hepatic steatosis with reduced insulin resistance, ALT levels and serum interleukin 6 (IL-6) levels were associated with voluntarily or electrically contracted quadriceps and hamstrings, independent of changes in muscle mass (53). A short-term AT program decreased serum markers of hepatic apoptosis in a small sample of obese NAFLD patients. Fealy et al (33) proposed that changes in the proapoptotic environment following short-term exercise are mediated by improved insulin sensitivity and increased oxidative capacity.

Fetuin-A is a liver protein believed to be associated with NAFLD and type 2 diabetes. Seven days of AT reduced insulin resistance (P<0.05) and circulating fetuin-A levels (P<0.02) in obese adults with clinically diagnosed NAFLD, with a correlation between these two parameters (P=0.04). These occurred independent of changes in body weight. This study suggests that improvements in glucose tolerance in patients with NAFLD after physical exercise may be mediated by lowering fetuin-A levels, particularly in skeletal muscles (40).

ADIPOKINES AS BIOMARKERS OF NASH AND OBESITY IN ADOLESCENTS

Noninvasive routine laboratory tests in NAFLD patients have been described in recent reviews (54,55) and a large study (56). In the present work, we focus on serum adipokines levels. Adipokines, such as adiponectin, leptin and ghrelin, were measured in samples of obese children and adolescents, with or without NAFLD. These findings are presented in Table 3 (32,57-61).

The main adipokines studied in NAFLD are adiponectin, leptin and ghrelin. Differences in adiponectin levels between controls and NAFLD patients are generally small. Among NAFLD patients, adiponectin levels are generally decreased in NASH compared with simple steatosis, such that decreased adiponectin levels can be used to predict NASH progression (62). On the other hand, leptin levels are higher in NAFLD patients compared with controls (57). Blood leptin levels reflect total body fat, and correlate with fibrosis and insulin resistance (63).

Adiponectin, resistin and retinol-binding protein-4 could be used to differentiate between steatosis with elevated serum ALT levels and non-steatotic obese patients. Adiponectin and resistin were significantly lower and retinol-binding protein-4 was significantly higher in obese children with advanced steatosis compared with obese children without liver steatosis. Leptin was not a good predictor of liver steatosis or hepatopathic obesity (57).

Adiponectin levels were negatively correlated with the NAFLD activity score in a recent meta-analysis (64). In this study, low adiponectin levels were associated with NAFLD progression to NASH, with nonsignificant differences in adiponectin levels between healthy controls and patients with simple steatosis. In contrast, leptin levels were elevated in NAFLD patients compared with nonsteatotic controls in a sample of obese children (57). Elevated leptin levels could further be used to predict significant fibrosis (P<0.02), and classify NAFLD patients according to the level of fibrosis (65).

The relationship between interdisciplinary therapy consisting of various forms of physical exercise, clinical, nutritional and psychological interventions, and changes in serum adipokines levels was assessed in samples of obese adolescents. Improvements in the levels of noninvasive biomarkers and clinical characteristics of disease progression were observed, particularly among patients with NAFLD receiving multidisciplinary intervention (32,61).

USE OF INTRAGASTRIC BALLOON AND CHANGES IN ADIPOKINE LEVELS

Combining the use of intragastric balloon with physical exercise led to more substantial weight loss, especially fat mass, in obese patients (66). Aside from weight loss, use of intragastric balloon (Bioenteric, Inamed Health, USA) led to improvement in the metabolic syndrome parameters such as diabetes mellitus, hypertension, dyslipidemia and fatty liver in compliant patients (67). After six months of intragastric balloon placement, 77.5% of subjects no longer met the diagnostic criteria for the metabolic syndrome in a sample of 40 previously overweight or obese patients with the metabolic syndrome. This procedure further reduced the percentage of truncal, android, gynoid and total fat (P=0.0001), and improved lung function parameters (68). In a small sample of obese patients treated with the BioEnterics intragastric balloon, the amount of weight loss experienced during the first month may be an important predictor of weight loss up to one year after balloon removal (18 months since baseline) (69). Table 4 describes the consequence of intragastric balloon placement in obese patients with NAFLD (70-73). Compared with baseline, anthropometric and biochemical measurements were improved at the time of balloon removal.

Studies show that weight loss is associated with histological improvements in obese NAFLD patients. The BioEnterics intragastric balloon has shown promising results in terms of weight loss in morbidly obese individuals and in individuals with treatment-resistant obesity. The balloon is filled with fluid to provide a sensation of satiety, thus reducing food ingestion during the time the balloon is used. Furthermore, it was conjectured that the short-term presence of the intragastric balloon would create behavioural routine whereby lower food amounts are consumed even after the balloon is removed, usually within six months of placement (70).

BioEnterics intragastric balloon placement was associated with improved eating patterns (74). Treatment with intragastric balloon for six months followed by 12 months of behavioural modification was associated with more pronounced weight loss and reversal of the metabolic syndrome compared with 12 months of behavioural modification alone in a small sample of obese adults (75). A reduction in hunger and an increase in sense of satiety was observed among nine obese children using a gastric balloon system (Obalon, Obalon Therapeutics, USA) for three months (73).

Leptin

Treatment with intragastric balloon placement, low-calorie diet (1500 kcal/day) and physical exercise was associated with decreased leptin levels, which correlated with weight loss (76). There were significant changes in leptin levels in both patient groups throughout the 12-month study period in a sample of 43 obese Caucasian patients.
TABLE 3

Adipokine levels in children and adolescents

<table>
<thead>
<tr>
<th>Study and sample population</th>
<th>Changes in adipokines</th>
<th>Associations and diagnostic performance</th>
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<tbody>
<tr>
<td>de Piano et al (32)</td>
<td>Mean adiponectin: 2.7±0.7 in group I, 2.5±0.4 in group II and 4.7±1.1 μg/mL in group III (P&lt;0.001 vs group I and P=0.001 vs group II)</td>
<td>Adiponectin: sensitivity of 84.21% and specificity of 63.64% for advanced liver steatosis at cut-off 2.56 μg/mL</td>
</tr>
<tr>
<td>Klein et al (60)</td>
<td>Mean adiponectin: 12.2±4.9 μg/mL</td>
<td>Resistin: sensitivity of 36.8% and specificity of 95.5% for advanced liver steatosis at cut-off 5.2 ng/mL</td>
</tr>
<tr>
<td>AT group: 15 patients without NAFLD and 14 with NAFLD</td>
<td>Mean leptin: 27.4±11.9 in group I, 27.4±11.9 in group II and 27.4±11.9 μg/mL in group III (P=0.560 vs group I and P=0.681 vs group II)</td>
<td>Resistin: sensitivity of 100% and specificity of 77.65% to differentiate hepatopathic obese children at cut-off 12.0 ng/mL</td>
</tr>
<tr>
<td>AT + RT group: 15 patients without NAFLD and 14 with NAFLD</td>
<td>Mean leptin: 27.4±11.9 in group I, 27.4±11.9 in group II and 27.4±11.9 μg/mL in group III (P=0.560 vs group I and P=0.681 vs group II)</td>
<td>RBP4: sensitivity of 84.20% and a specificity of 68.20% for advanced liver steatosis at cut-off 35 μg/mL</td>
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</table>

Koot et al (58) 119 severely obese children with a BMI of 40 kg/m². Leptin levels were lower at baseline and fell following meals. However, in obese individuals, ghrelin (divided into morbidly and nonmorbidly obese based on a BMI cut-off of 40 kg/m²) (77). Throughout the study period, leptin levels remained below baseline values. Leptin levels decreased beginning at month 1 (mean 21.1 ng/mL versus mean 28.7 ng/mL at baseline), with slight oscillations thereafter (mean 17.4 ng/mL to 19.0 ng/mL) among morbidly obese individuals (BMI ≥240 kg/m²). Leptin levels were lower at months 1 and 6 (mean 11.8 ng/mL and 10.5 ng/mL, respectively), versus mean 25.1 ng/mL at baseline) among obese individuals (BMI >40 kg/m²), with a slight rise approaching month 12 (mean 17.5 ng/mL) (77). Leptin significantly decreased during the first month in small samples of obese patients undergoing intragastric balloon placement (median 67.1 ng/dL at baseline, 57.1 at month 1, 39.3 at month 3 and 26.5 ng/dL at month 6; P=0.0353) (78). Leptin levels decreased throughout the study period in 22 obese individuals who were treated with the BioEnterics intragastric balloon, low-calorie diet (1500 kcal/day) and physical exercise, or low-calorie diet and physical exercise alone (76). Leptin levels decreased throughout the study period in a small sample of nonmorbidly obese patients with intra gastric balloon (27.8±3.7 ng/mL at baseline versus 18.7±2.7 ng/mL at month 6; P=0.024) (79). Leptin was significantly decreased (30.4±17.2 μg/L versus 14.9±15.5 μg/L; P<0.001) after six months in 22 obese individuals with the balloon (80).

Ghrelin
Ghrelin is a peptide hormone that stimulates food intake. Abnormalities in meal-related peptides, such as ghrelin, are associated with binge eating disorders (81). In normal individuals, ghrelin levels rise before meals and fall following meals. However, in obese individuals, ghrelin levels remained relatively stable in the control group among obese patients treated with the BioEnterics intragastric balloon, low-calorie diet (1500 kcal/day) and physical exercise, or low-calorie diet and physical exercise alone (76). Leptin levels decreased throughout the study period in a small sample of nonmorbidly obese patients with intragastric balloon (27.8±3.7 ng/mL at baseline versus 18.7±2.7 ng/mL at month 6; P=0.024) (79). Leptin was significantly decreased (30.4±17.2 μg/L versus 14.9±15.5 μg/L; P<0.001) after six months in 22 obese individuals with the balloon (80).
levels are lower than in healthy individuals, a phenomenon believed to be the result rather than the cause of overeating (81). Obesity was shown to actually lead to lower ghrelin levels. Ghrelin significantly increased (240.5±101.5 μg/L versus 335.8±149.2 μg/L; P<0.002) after six months in 22 obese individuals with intragastric balloon treatment (80). Among obese individuals (BMI <40 kg/m²), ghrelin levels peaked around month 3 (mean 1346.2 pg/mL versus mean 958.3 pg/mL at baseline), and then decreased steadily to near baseline levels by month 12 (mean 922.6 pg/mL). Ghrelin levels remained relatively stable throughout the first six months of the study among morbidity obese individuals (BMI ≥40 kg/m²), with a drop toward month 12 (mean 742.6 pg/mL versus mean 498.3 pg/mL at baseline) (77).

Blood ghrelin levels were measured every 15 min for 1 h after breakfast at the start of treatment, after 13 weeks and after 26 weeks in a sample of 40 obese patients randomly assigned to a 13-week period of low-calorie diet (1500 kcal) and physical exercise, followed by 13 weeks of balloon. There were no differences in plasma ghrelin levels (either fasting or trough levels) between groups. Ghrelin levels did not change from baseline to end of treatment (mean fasting 725.4±113.8 pg/mL and trough 596.0±105.3 pg/mL at baseline versus mean fasting 773.4±113.8 pg/mL and trough 669.3±128.4 pg/mL at six months) despite weight loss (82).

Weight loss was more pronounced in the balloon group at six months (17.1±8.0 kg versus 3.2±6.4 kg) in a sample of 21 obese subjects who were treated with a BioEnterics intragastric balloon, low-calorie diet (1500 kcal) and physical exercise, and 15 controls treated with low-calorie diet and physical exercise alone. In the balloon group, ghrelin levels increased at one month (P=0.006 versus baseline) and slowly decreased thereafter until they approached baseline levels at three months after balloon removal. Ghrelin levels were relatively stable throughout the study in the control group. Based on these findings, balloon treatment is associated with transiently elevated ghrelin levels in obese patients (76).

Weight reduction was correlated with plasma ghrelin levels in 17 nonmorbidly obese patients. Plasma ghrelin levels decreased throughout the study (3.2±0.4 ng/mL at baseline versus 1.9±0.1 ng/mL at month 6; P=0.021). In this study, weight loss associated with intragastric balloon correlated with plasma ghrelin variations (79). Ghrelin was significantly increased (240.5±101.5 μg/L versus 335.8±149.2 μg/L; P<0.002) after six months in 22 obese individuals with intragastric balloon placement (80).

Weight loss occurred in both groups in a randomized, double-blinded, sham-controlled trial of four months’ duration in morbidly obese patients treated with either intragastric balloon placement or sham operation. However, there were no significant differences between the balloon group and the control group in terms of degree of weight loss. These changes were not mediated by changes in plasma ghrelin levels, because ghrelin levels did not fluctuate between fasting and postprandial conditions, and between the two treatments (mean fasting ghrelin 934.4±199.2 pg/mL at baseline versus 947.1±195.1 pg/mL at day 30 in the balloon group and mean fasting ghrelin 970.1±125.2 pg/mL at baseline versus 962.0±93.9 pg/mL at day 30 in the control group) (83).

**Adiponectin**

Adiponectin levels remained unchanged throughout the study in a sample of obese patients treated with intragastric balloon, low-calorie diet (1500 kcal) and physical exercise (76). Adiponectin levels showed no significant difference in other small samples of obese patients undergoing intragastric balloon placement (78,80). A transient increase in adiponectin levels was observed in obese controls treated with low-calorie diet (1500 kcal) and physical exercise without intragastric balloon placement (P=0.045 at six months versus baseline) (76). Adiponectin levels did not change significantly in another study (78). In contrast, Mion et al (79) found increasing adiponectin levels (6.6±0.5 ng/mL at baseline versus 7.8±0.8 ng/mL at six months; P=0.037).

**BARIATRIC SURGERY AND CHANGES IN CIRCULATING LIVER ENZYME LEVELS, INFLAMMATORY MARKERS AND ADIPOKINE LEVELS**

An additional nonmedicinal intervention that can be used in NAFLD patients is bariatric surgery. Bariatric surgery is believed to ameliorate some of the abnormalities associated with NAFLD and, thus, lead to an improvement in NAFLD. This is believed to involve improvements in circulating liver enzyme levels, decreases in inflammatory marker levels, and changes in adipokines levels such as increases in adiponectin levels.
and decreases in leptin (84,85). Improvements in steatosis, lobular inflammation, chronic portal inflammation and steatohepatitis were noted in a sample of NAFLD patients in which liver biopsy were analyzed pre- and post-bariatric surgery (86). Roux-en-Y gastric bypass surgery was shown to decrease the metabolic syndrome parameters, as well as the prevalence of comorbidities associated with obesity, including fatty liver and steatosis. This effect was observed in both sexes and among all individuals 18 to 65 years of age (87). The effects of bariatric surgery in obese populations, with or without NAFLD/NASH, are described in Table 5 (88-97).

Liver enzymes

The effects of bariatric surgery on circulating levels of liver enzymes were assessed in several studies (88-93). ALT, AST and gamma-glutamyl transpeptidase (γ-GTP) levels are generally lower at follow-up (six to 12 months after the surgical intervention) compared with baseline levels (ie, before surgery) in samples of obese patients (90,93,98). Lower serum AST and ALT levels were maintained at two and 10 years follow-up in a large sample of obese subjects who underwent bariatric surgery. ALT reduction was proportional to weight loss (99). In another study, serum AST and ALT levels were maintained at two and 10 years follow-up in samples of obese patients compared with nonobese controls. Following bariatric surgery in one study (97). Significant decreases in TNF-α levels at baseline were observed in a sample of obese patients who underwent laparoscopic sleeve gastrectomy and diet changes (127.5±69.6 ng/L at baseline, 62.6±60.9 ng/L at six months and 87.5±59.1 ng/L at 12 months) (105). Mean serum visfatin levels decreased at six weeks’ follow-up in a small sample of severely obese patients undergoing gastric bypass (94). Serum resistin levels were elevated at six months, but decreased to below baseline levels by 12 months after laparoscopic adjustable gastric banding (93).

Inflammatory markers

Elevated mean blood high-sensitivity CRP and interleukin (IL)-6 levels were present at baseline among obese patients versus lean controls (CRP: 26.19±23.17 g/L versus 1.71±2.27 g/L, respectively; P<0.001, and IL-6: 3.84±1.67 pg/mL versus 0.92±0.47 pg/mL, respectively; P<0.001). IL-6 and high-sensitivity CRP levels decreased by three months after bariatric surgery (97). Significant decreases in CRP levels were observed after six months in a sample of obese patients undergoing laparoscopic adjustable gastric banding. These differences were even more pronounced at 12 months (93). Serum IL-6 levels decreased within six months of bariatric surgery in a small sample of severely obese patients (96). Elevated mean TNF-α levels at baseline were observed in a sample of morbidly obese patients compared with lean controls (2.10±1.86 pg/mL versus 0.86±0.67 pg/mL; P<0.001). TNF-α levels initially increased at three months, but stabilized to near-baseline levels by month 12 after bariatric surgery in one study (97). Significant decreases in TNF-α were observed after six months elsewhere (93). Serum TNF-α levels were undetectable in another study, while hepatic TNF-α messenger RNA expression was decreased at six months (106). IL-18, soluble tumour necrosis factor (TNF) receptor 2 and CRP levels decreased at 12 months’ follow-up compared with baseline in a small sample of morbidly obese subjects undergoing gastric bypass (96).

FRUCTOSE

Fructose is found in a wide variety of processed foods and beverages (107). A relationship is believed to exist between high fructose consumption and NAFLD development and progression. While providing fructose as a dietary supplement for seven days in children of type 2 diabetes patients or controls, Lê et al (108) observed that a diet high in fructose is associated with dyslipidemia, especially in individuals with a family history of type 2 diabetes. A high-fructose diet significantly increased intrahepatocellular lipids, intramyocellular lipids, very low-density lipoprotein-triacylglycerols and fasting hepatic glucose output in both groups. However, higher intrahepatocellular lipids and total triacylglycerols, and lower whole-body insulin sensitivity occurred in children with a family history of type 2 diabetes (108). High acute fructose consumption from soft drinks further led to elevated endotoxin levels, which are associated with the presence of hepatic steatosis. High chronic fructose consumption was associated with persistently elevated endotoxin levels, suggesting that the association between fructose consumption and liver steatosis is mediated, at least in part, by endotoxin (109).
### TABLE 5
Circulating liver enzyme levels, adipokines and inflammatory markers in patients undergoing bariatric surgery

<table>
<thead>
<tr>
<th>Study; sample population</th>
<th>Changes following bariatric surgery</th>
</tr>
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<tbody>
<tr>
<td>Vargas et al (88); 26 morbidly obese patients (96.1% had NASH) undergoing Roux-en-Y gastric bypass with a modified Fobi-Capella technique (follow-up after 16.3±3 months)</td>
<td>Mean (± SD) serum ALT: 30.7±17.1 mg/dL at baseline vs 20.7±7.2 mg/dL at month 12 (P&lt;0.0001)</td>
</tr>
<tr>
<td>Cazzo et al (89); 63 obese subjects undergoing Roux-en-Y gastric bypass surgery</td>
<td>Mean serum AST: 25.7±10.6 mg/dL at baseline vs 21.2±5.4 mg/dL at month 12 (P=0.0005)</td>
</tr>
<tr>
<td>Carazo et al (90); 60 morbidly obese patients undergoing bariatric surgery</td>
<td>Mean (± SD) plasma AST: 29.8±3.3 IU/L before surgery vs 20.4±1.0 IU/L at month 12 (P=0.0070)</td>
</tr>
<tr>
<td>Tai et al (91); 21 morbidly obese patients undergoing Roux-en-Y gastric bypass</td>
<td>Median serum AST: 27.0 IU/L before surgery vs 27.0 IU/L at month 12 (P=0.66)</td>
</tr>
<tr>
<td>Felipo et al (92); 47 morbidly obese patients undergoing bariatric surgery (evaluated before and 18±5 months after surgery)</td>
<td>Mean serum ALT: 17±5.0 IU/L in controls, 31±17 IU/L in simple steatosis patients before surgery, 23±9.5 IU/L in simple steatosis patients after surgery, 24±16 IU/L in NASH patients before surgery and 20±26 IU/L in NASH patients after surgery</td>
</tr>
<tr>
<td>Neuman et al (93); 30 severely obese patients undergoing laparoscopic adjustable gastric banding</td>
<td>Mean serum γ-GTP: 34.5±4.0 IU/L at baseline, 26.1±2.4 IU/L at month 6 (P=0.05) and 20.8±1.6 IU/L at month 12 (P=0.01)</td>
</tr>
<tr>
<td>Hosseinzadeh-Attar et al (94); 35 severely obese patients undergoing gastric bypass</td>
<td>Mean serum γ-GTP: 40±10 IU/L in controls, 39±26 IU/L in simple steatosis patients before surgery, 19±15 IU/L in simple steatosis patients after surgery (P&lt;0.05 vs before surgery), 45±19 IU/L in NASH patients before surgery and 23±11 IU/L in NASH patients after surgery (P&lt;0.05 vs before surgery)</td>
</tr>
<tr>
<td>Machado et al (95); 82 morbidly obese individuals with biopsy-proven NAFLD (13.4% had NASH) undergoing bariatric surgery</td>
<td>Mean serum ALT: 20.1±6.9 ng/mL overall, 22.2±6.8 ng/mL in NASH and 19.1±6.9 ng/mL in no NASH (P=0.014 vs NASH)</td>
</tr>
<tr>
<td>Villarrasa et al (96); 65 morbidly obese subjects undergoing gastric bypass</td>
<td>Median serum adiponectin: 11±4 i µg/mL n controls, 7±2 µg/mL in simple steatosis patients before surgery (P&lt;0.05 vs control), 16±10 µg/mL in simple steatosis patients after surgery (P&lt;0.001 vs before surgery), 6±2 µg/mL in NASH patients before surgery (P&lt;0.05 vs control) and 12±7 µg/mL in NASH patients after surgery (P=0.008 vs before surgery)</td>
</tr>
<tr>
<td>Illán-Gómez et al (97); 60 morbidly obese women undergoing gastric bypass 30 lean controls</td>
<td>Mean serum leptin: 13±9 ng/mL before surgery vs 24±11 ng/mL in NASH patients after surgery (P&lt;0.01 vs before surgery)</td>
</tr>
</tbody>
</table>

**ALT** Alanine aminotransferase; **AST** Aspartate aminotransferase; **CRP** C-reactive protein; **γ-GTP** Gamma-glutamyl transpeptidase; **hsCRP** High-sensitivity CRP; **IL** Interleukin; **NAFLD** Nonalcoholic fatty liver disease; **NASH** Nonalcoholic steatohepatitis; **sTNFR** Soluble tumour necrosis factor receptor; **vs** Versus
The association between elevated fructose consumption and NAFLD risk appears to be influenced by the actual amount of fructose consumed. Higher fructose intake (highest intake quartile: 29.2 g/day to 88.0 g/day) was not associated with NAFLD in an older Finnish population compared with lower fructose intake (lowest intake quartile: 2.2 g/day to 15.2 g/day). A possible explanation for these findings is that the levels of fructose intake were similar to the average population levels (110).

Because lifestyle interventions such as physical activity and dietary modifications represent an important first line of treatment in NAFLD patients, limiting fructose intake is an important area of research (111). Decreased fructose intake led to decreased intrahepatic fat content in a small sample of NAFLD patients at six months. However, this was coupled with reduced intake of glucose and sucrose, as well as reduced overall carbohydrate intake and energy consumption, such that no definitive conclusion can be drawn with regards to decreasing fructose alone (112).

In a recent study, consuming isocaloric diets with high fructose or high glucose content did not cause significant changes in the hepatic concentration of triacylglycerols or the serum levels of liver enzymes in a sample of healthy overweight men, with no differences between treatments. However, when high fructose or high glucose was administered as part of a hypercaloric diet, significant increases in these parameters occurred. This study suggests that overnutrition is associated with NAFLD risk factors, and not the levels of specific macronutrients (113). These findings are corroborated by a recent systematic review and meta-analysis (114) that showed no clear evidence that markers of hepatotoxicity are associated with excessive fructose intake per se, but rather with excessive energy intake. A separate systematic review and meta-analysis in controlled feeding trials shows that deriving a higher percentage of energy from fructose is not associated with a higher NAFLD risk in healthy controls. High fructose consumption coupled with excess energy intake is associated with elevated intrahepatic lipids and ALT levels, again suggesting that it is overnutrition rather than overconsumption of fructose that predisposes healthy individuals to NAFLD (115). Because restricting caloric intake, as a whole, is one of the main interventions aimed at weight loss in NAFLD patients, it is unclear what benefit, if any, would lower fructose intake have in the absence of lower overall energy intake (116).

Obesity was a risk factor for NAFLD at 17 years of age in a large sample of adolescents followed since birth. A higher energy-adjusted fructose intake at 14 years of age was associated with a higher risk of NAFLD at 17 years of age among obese adolescents, and this association was maintained after adjusting for confounding variables. This study shows that fructose rather than total sugar consumption is a risk factor for developing NAFLD in obese adolescents (117). Decreasing fructose intake reduced markers of liver dysfunction in a small sample of NAFLD children and adolescents (118). Sullivan et al (119) show in a small sample of NAFLD, obese controls and lean controls that fructose absorption and metabolism was more effective between NAFLD patients and lean control, but similar in NAFLD and obese controls. Children with NAFLD were more sensitive to dyslipidemia occurring in response to dietary fructose intake than children without NAFLD (120). While fructose alone is not generally believed to cause NAFLD development, fructose metabolites may influence NAFLD progression, and fructose intake may favour NAFLD progression on a background of pre-existing risk factors such as obesity, the metabolic syndrome or diabetes (121,122). In view of the continued increase of the number of cases of NAFLD-NASH, Chalasani et al (123) published the practice guidelines of the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association.

CONCLUSIONS

The current practice for diagnosis of patients with NASH is to perform a liver biopsy and imaging. The present review defines novel criteria based on noninvasive biomarkers that optimized noninvasive diagnosis of liver disease in patients with NAFLD. Active inflammation of the liver (steatohepatitis) should be excluded first by blood tests that should assess adipokine and pro-inflammatory cytokine levels.

The clinical evaluation should also involve a personalized evaluation of laboratory data. Monitoring adipokine levels may inform the clinician on the changes in the severity of the liver disease in time as well as of the efficacy of the intervention. Additionally, we suggest that these biomarkers may also be useful in improving the assessment of noninvasive fibrosis.

Dietary habits may promote steatohepatitis directly by modulating hepatic triglyceride accumulation and antioxidant activity, as well as indirectly by affecting insulin sensitivity and postprandial triglyceride metabolism. Our findings provide further rationale for more specific alimentary and physical activity interventions, particularly in nonobese, nondiabetic, normolipidemic NASH patients. In addition, the review highlights the importance of other procedures, such as gastric balloon and bariatric surgery, in improving outcomes. Finally, we recommend continued collaboration between clinicians and laboratory, which will benefit patients.

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25. Neuman et al


Nonmedicinal interventions in NAFLD


